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HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

OFFICE OF
PREVENTION, PESTICIDES, A
TOXIC SUBSTANCES

MEMORANDUM

DATE: 9-NOV-2007

SUBJECT: PP#: 6F7115. Difenoconazole in/on Fruiting Vegetables, Pome Fruit, Sugar Beets, Tuberous and Corm Vegetables, and Imported Papaya. **Health Effects Division (HED) Revised Risk Assessment.** DP#: 346591. PC Code: 128847. Decision#: 371264.

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THROUGH: P.V. Shah, Ph.D., Branch Senior Scientist
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Fungicide Branch (FB)/Registration Division (RD); (7505P)

Note: This document supercedes the previous difenoconazole risk assessment (M. Sahafeyan, D333320, 9-AUG- 2007).

The HED of the Office of Pesticide Programs (OPP) is charged with estimating the risk to human health from exposure to pesticides. The RD of OPP has requested that HED evaluate hazard and exposure data and conduct dietary, occupational, residential, and aggregate exposure assessments, as needed, to estimate the risk to human health that will result from all registered and proposed uses of difenoconazole (1-[2-[2-chloro-4-(4-chlorophenoxy)phenyl]-4-methyl-1,3-dioxolan-2-ylmethyl]-1*H*-1,2,4-triazole). A summary of findings is provided in this document. The risk assessment was provided by Mohsen Sahafeyan of RAB1; the hazard characterization was provided by William Greear; the residue chemistry review and dietary exposure assessment were provided by William Wassell and Mohsen Sahafeyan; the occupational/residential exposure and risk assessment was provided by Mark Dow; and the drinking water assessment was provided by Iwona Maher of the Environmental Fate and Effects Division (EFED).

NOTE: HED completed a Section 3 risk assessment for the use of difenoconazole in/on

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barley, cotton, sweet corn, and imported grapes and pome fruit (Memo, Levy, *et al.*, 05-AUG-05; DP# 319944). This document contains only those aspects of the risk assessment which are affected by the addition of the proposed difenoconazole uses.

Recommendation for Tolerances and Registration

Provided the petitioner submits revised Sections B and F, HED concludes that the residue chemistry and toxicology database are sufficient for conditional registration and establishment of permanent tolerances for residues of difenoconazole [1-[2-[2-chloro-4-(4-chlorophenoxy)phenyl]-4-methyl-1,3-dioxolan-2-ylmethyl]-1*H*-1,2,4-triazole] in or on the following commodities:

Fruit, Pome Group 11[1]	1.0 ppm
Vegetable, Fruiting, Group 8	0.60 ppm
Vegetable, Tuberous and Corm, subgroup 1C	0.01 ppm
Beet, sugar	0.01 ppm
Papaya	0.30 ppm
Apple, wet pomace	4.5 ppm
Beet, sugar, dried pulp	1.9 ppm
Potato, processed waste	0.04 ppm

[1] A tolerance without U.S. registration currently exists for residues of difenoconazole in/on pome fruit at 0.10 ppm.

Additionally, HED has determined that tolerances are needed for residues of difenoconazole [1-[2-[2-chloro-4-(4-chlorophenoxy)phenyl]-4-methyl-1,3-dioxolan-2-ylmethyl]-1*H*-1,2,4-triazole] and its metabolite CGA-205375 [1-[2-chloro-4-(4-chloro-phenoxy)phenyl]-2-[1,2,4]triazol-1-yl-ethanol] in or on the following commodities:

Milk	0.01 ppm
Meat[1]	0.05 ppm
Meat byproduct (except liver)[1]	0.10 ppm
Fat[1]	0.10 ppm
Liver[1]	0.20 ppm
Eggs	0.10 ppm

[1] Of cattle, hog, goat, horse and sheep.

Based on the submitted studies, HED also concluded that the currently-established tolerances for secondary residues in poultry, meat, fat and meat byproducts should be removed.

HED recommends that conversion of conditional registration to unconditional registration may be considered upon submission of the following residue chemistry data:

- It has been determined that CGA 205375 should be included in the tolerance expression for livestock commodities. The submitted liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS) method, REM 147.07, will be adequate for enforcement purposes. However, the petitioner should submit a revised method which includes a second MS/MS ion transition as specified by ACB and supply, with replenishment when requested, a standard of

metabolite CGA205375 to EPA National Pesticide Standard Repository at ACB laboratory (memo, Charles J. Stafford, ACB Project # B07-26, 10/2907).

- Data should be submitted depicting the stability of residues of difenoconazole and CGA 205375 in milk and cattle tissues during frozen storage for up to 10 months for milk and 9 months for tissues. The studies cited by the petitioner (report numbers ABR-93012 and 202/99), which contain storage stability data for difenoconazole and CGA 205375, should be submitted.

- Data should be submitted depicting the stability of residues of difenoconazole and CGA 205375 in egg and poultry tissue samples during frozen storage for up to 7 months for egg and 6 months for tissue samples. The studies cited by the petitioner (report numbers ABR-93012 and 202/99), which contain storage stability data for difenoconazole and CGA 205375, should be submitted.

- Storage stability data for residues of 1,2,4-traizole (T), triazole alanine (TA) and triazole acetic acid (TAA)] have not been submitted in conjunction with the subject petitions. However, storage stability data for these compounds have been requested as part of the Human-Health Aggregate Risk Assessment for 1,2,4-T, TA and TAA (M. Doherty, *et al.,* DP#322215, 2/7/06). Submission of the data requested in the 2/7/06 document will satisfy the storage stability data requirement for the subject petitions.

- Two additional tomato (cherry tomato or varieties of tomatoes in which the mature fruits will be less than 2 inches in diameter) field trials should be conducted in Zone 3.

- The petitioner should submit new confined rotational crop studies reflecting application of [^{14}C]difenoconazole, labeled in the phenyl and triazole rings, at 0.46 lb ai/A (1x the proposed maximum seasonal rate to annual crops). The studies should be conducted according to the requirements specified in OPPTS 860.1850.

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1.0 EXECUTIVE SUMMARY

Difenoconazole is a broad-spectrum fungicide with registered seed treatment (on domestic cereal grains and canola and foreign rye) and foliar treatment (on foreign banana, grapes and pome fruits) uses. Difenoconazole tolerances have been established in 40 CFR §180.475 for plant and livestock commodities and are expressed in terms of difenoconazole *per se*. Syngenta has petitioned the Agency to establish tolerances resulting from domestic foliar use of difenoconazole on fruiting vegetables, pome fruit, sugar beets, tuberous and corm vegetables and foreign foliar use of papaya (grown in Brazil) in addition to ornamental use (homeowner application potential).

Hazard Assessment

The toxicological database for difenoconazole is adequate to support Section 3 registration and permanent tolerances. There are no toxicology data gaps.

Difenoconazole possesses low acute toxicity by the oral, dermal and inhalation routes of exposure. It is not considered to be an eye or skin irritant and is not a sensitizer. In an acute neurotoxicity study in rats, reduced fore-limb grip strength was observed on day 1 in males and clinical sign of neurotoxicity in females at the limit dose of 2000 mg/kg. This effect in males is considered as transient since it was not observed at later observation points and high toxicity in females. In a subchronic neurotoxicity study in rats decreased hind limb strength was observed only in males, which was considered as nonspecific in nature. Therefore, difenoconazole is not considered neurotoxic compound. It is not mutagenic. It is not a developmental or reproductive toxicant. No systemic toxicity was observed at the limit dose in a 28-day dermal toxicity study in rats. A dermal absorption of 15.3% was observed through rat skin using an *in vivo* method. Chronic effects in the rat study are seen as cumulative decreases in body weight gains. Evidence for carcinogenicity was seen in only one species, mice, where liver tumors were induced at doses which were considered to be excessively high for carcinogenicity testing. No evidence of carcinogenicity was seen in rats.

Chronic feeding studies in mice showed decreased body-weight gains in male and female mice at termination. Treatment-related non-neoplastic lesions were confined to the liver and were supported by the clinical chemistry data at a level of 300 ppm (46.29 and 57.79 mg/kg/day for males and females, respectively). Liver tumors were observed in mice at 300 ppm and higher; however, based on the excessive toxicity observed at the two highest doses of 2500 and 4500 ppm (females terminated after two weeks due to excessive toxicity resulting in moribundity and death), the absence of tumors at the two lower doses of 10 and 30 ppm and the absence of genotoxic effects, HED's Cancer Peer Review Committee (CPRC) recommended for a cancer classification of C (**possible human carcinogen**). A margin-of-exposure (MOE) approach in risk assessment was advocated by the CPRC utilizing the no-observable-adverse-effects-level (NOAEL) of **30** ppm (**4.7** and **5.6** mg/kg/day in males and females, respectively) and the lowest-observable-adverse-effects-level (LOAEL) of **300** ppm (**46.3** and **57.8** mg/kg/day in males and females, respectively) from the mouse study using only those biological endpoints which were related to tumor development (*i.e.*, hepatocellular hypertrophy, liver necrosis, fatty changes in the liver and bile stasis) (Memo, Jess Rowland and Esther Rinde, 27-JUL-1994; Memo, PV Shah, 1-

March-2007, HED Doc. No. 005453).

Dose-Response Assessment

On 08-SEP-1998, HED's Hazard Identification Assessment Review Committee (HIARC) evaluated the toxicology data base of difenoconazole and re-assessed the RfD established in 1994, as well as the toxicological endpoints for the dietary and occupational exposure risk assessments that were selected in 1994. At this meeting, the HIARC also addressed the potential enhanced sensitivity of infants and children from exposure to difenoconazole as required by the Food Quality Protection Act (FQPA) of 1996 (HED Doc. No. 012873, 25-SEP-1998). In July 2007, RAB1 toxicologists and the risk assessment team reevaluated the endpoints selected by the HIARC since new studies were submitted. RAB1 toxicologists and the risk assessment team also reevaluated the FQPA assessments. The risk assessment team concluded that the default 10x FQPA Safety Factor (SF) be reduced to 1x when assessing dietary and residential exposures based on:

- completeness of the toxicological database for difenoconazole;
- developmental toxicity studies showed no increased sensitivity in fetuses as compared to maternal animals following *in utero* exposures in rats and rabbits, and pre/post natal exposure in the two generation reproduction toxicity study in rats;
- no evidence of abnormalities in the development of the fetal nervous system in the pre/post natal studies;
- the developmental neurotoxicity study is not required; and
- there are no residual uncertainties in the toxicology database.

The toxicological doses relevant to this assessment are summarized below. It should be noted that a cancer dietary assessment was not conducted for difenoconazole because the cancer NOAEL is higher than the chronic NOAEL; therefore, the chronic dietary risk estimate is more protective.

Acute dietary (general population including infants and children)	NOAEL = 25 mg/kg/day	acute Rfd[3] and acute population-adjusted dose (PAD) = 0.25 mg/kg/day
Chronic dietary	NOAEL = 0.96 mg/kg/day	chronic Rfd and cPAD[4] = 0.01 mg/kg/day
Short-term dermal[1]	oral NOAEL = 1.25 mg/kg/day	Target MOE[5] ≥ 100 (occupational and residential)
Short-term inhalation[2]	oral NOAEL = 1.25 mg/kg/day	Target MOE ≥ 100 (occupational and residential)

[*] Dermal absorption factor = 15.3%
[#] Inhalation absorption factor = 100%
[3] RfD = Reference Dose
[4] cPAD = chronic PAD
[5] MOE = margin of exposure

Occupational and Residential Exposure and Risk Assessments

The occupational and residential exposure assessment addresses the use of the product Inspire® Fungicide. Inspire® is not a currently registered product. Inspire® is a liquid formulation which contains 2.08 lb ai difenoconazole per gallon.

Based on the proposed uses (fruiting vegetables, pome fruit, root and tuberous vegetables, sugar beets, ornamental foliar treatment) of difenoconazole, the potential for residential and occupational exposures exists. HED believes pesticide handlers will be exposed to short-term duration (1 - 30 days) exposures, but not to intermediate-term (1 - 6 months) duration exposures. Moreover, since the short-term and intermediate-term toxicological endpoints are the same, the assessment of short-term exposure and risk is adequate to describe risk from an intermediate-term exposure, should that occur.

Residential- A MOE of 100 is adequate to protect residential pesticide handlers from short-term/intermediate-term dermal and inhalation exposures to difenoconazole. MOEs are >100; therefore, residential exposures are not of concern to HED.

Occupational- A MOE of 100 is adequate to protect occupational pesticide handlers from exposures to difenoconazole. Provided occupational handlers wear protective gloves, all MOEs are >100; therefore occupational exposures are not of concern to HED.

Post-Application- A MOE of 100 is adequate to protect agricultural workers from post-application exposures. The short-term duration MOE is <100 for floricultural activities and therefore is of concern; MOEs for all other crops are ≥100 and therefore, are not of concern. With respect to residential post-application exposures, current HED policy (see ExpoSAC minutes from 8/19/99 and 10/11/01) specifies that no significant post-application exposure is anticipated from ornamentals, either by residents or professional applicators; therefore, no residential postapplication assessment was conducted. Based on the requested use pattern, HED recommends changing the restricted entry level to 10 days to protect agricultural workers for floricultural activities. Use of the lower rate of application (0.03 lb ai/A), results in MOEs that are not of concern.

Drinking Water

Since HED does not have ground or surface water monitoring data to calculate quantitative aggregate exposure, estimates of difenoconazole levels in surface and ground water were made using computer modeling. Estimated drinking water concentrations (EDWCs) were provided for both surface water model (Pesticide Root Zone/Exposure Analysis Modeling System (PRZM/EXAMS)) and groundwater model (Screening Concentration in Ground Water (SCI-GROW)) by EFED (Memo, I. Maher, 19-JUN-2007, DP#333319 and 340041). The highest estimated EDWC in ground water, is from non-agricultural uses at **0.00128 µg/L (ppb)**. The highest estimated EDWC in surface water is from aerial applications of difenoconazole to California ornamental nurseries; the estimated surface water residues for 1-in-10 year annual

peak and 1-in-10 year annual mean are **13.3 and 9.43 µg/L (ppb)** respectively which are used in the acute and the chronic dietary risk assessments correspondingly. Generally, the uncertainties associated with modeling surface or ground water are not expected to substantially decrease the conservativeness of Tier II modeling results. Drinking water was incorporated directly into the dietary assessment.

Dietary Exposure Estimates

Acute and chronic dietary exposure risk assessments were conducted for the existing uses and proposed new uses of difenoconazole. A cancer dietary assessment was not conducted for difenoconazole because the cancer NOAEL is higher than the chronic NOAEL; therefore, the chronic dietary risk estimate is more protective.

The Dietary Exposure Evaluation Model - Food Consumption Intake Database (DEEM-FCID™, ver. 2.03) model was used, which incorporates consumption data from the United States Department of Agriculture's (USDA) Continuing Surveys of Food Intake by Individuals (CSFII), 1994-1996 and 1998. No monitoring data from USDA's Pesticide Data Program (PDP) or the Food and Drug Administration's (FDA) Surveillance Monitoring Program were available for difenoconazole.

The acute and chronic analyses for difenoconazole assumed tolerance-level residues, 100% crop treated (CT), and empirical and DEEM™ (ver. 7.76) default processing factors. The resulting acute food exposure estimates were not of concern (<100% of the acute population-adjusted dose (aPAD)) at the 95th percentile of the exposure distribution for the U.S. general population (2% aPAD) and all population sub-groups; the most highly exposed population subgroup was all-infants <1 year old with 8% aPAD. The resulting chronic food exposure estimates were not of concern (<100% cPAD) for the US general population (18% cPAD) and all population sub-groups; the most highly exposed population subgroup was children 1-2 years old with 56% cPAD.

The Agency have concern about potential toxicity to metabolites of difenconazole (1,2,4-T, TA and TAA) which are common metabolites of most of the triazole fungicides. The acute and chronic aggregate (food + water) dietary exposure analyses for 1,2,4-T, and TA+TAA from use of all registered and proposed triazole-based pesticides were updated in separate memorandums (M. Sahafeyan, DP#341803 and DP#344298) according to the HED recommendation (Memo, M. Doherty, *et al*, DP#322215, 7-FEB-2006) to include the new difenocoanzole proposed uses. These analyses indicate that the acute and chronic risk from dietary exposure to 1,2,4-T and TA+TAA from all registered and proposed triazole-based pesticides are not of concern.

The results of acute and chronic aggregate dietary exposure analysis for 1,2,4-T indicate that the highest aPAD is 32% for the all-infants population sub-group at the 95%ile of exposure distribution and the highest cPAD is 41% for children 1-2 years old, both below HEDs level of concern(<100% aPAD and <100% cPAD).

The results of acute and chronic aggregate dietary exposure analysis for TA+TAA indicate that the aPAD for females 13-49 years old (the only group with toxicological end point) is 28% at the

95th percentile of exposure distribution and the highest cPAD is 27% for children 1-2 years old, both below HEDs level of concern(<100% aPAD and <100% cPAD).

Exposure Scenarios and Risk Conclusions

Including all existing and proposed uses, human-health risk assessments have been conducted for the following exposure scenarios: chronic dietary exposures (food + water) + residential short-term exposure (dermal + inhalation). **The aggregate exposure and risk estimates are not of concern to HED.**

Recommendation for Tolerances and Registration

Provided the petitioner submits revised Sections B and F, HED concludes that the residue chemistry and toxicology database are sufficient for conditional registration and establishment of permanent tolerances for residues of difenoconazole [1-[2-[2-chloro-4-(4-chlorophenoxy)phenyl]-4-methyl-1,3-dioxolan-2-ylmethyl]-1*H*-1,2,4-triazole] in or on the following commodities:

Fruit, Pome Group 11[1]	1.0 ppm
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[1] A tolerance without U.S. registration currently exists for residues of difenoconazole in/on pome fruit at 0.10 ppm.

Additionally, HED has determined that tolerances are needed for residues of difenoconazole [1-[2-[2-chloro-4-(4-chlorophenoxy)phenyl]-4-methyl-1,3-dioxolan-2-ylmethyl]-1*H*-1,2,4-triazole] and its metabolite CGA-205375 [1-[2-chloro-4-(4-chloro-phenoxy)phenyl]-2-[1,2,4]triazol-1-yl-ethanol] in or on the following commodities:

Milk	0.01 ppm
Meat[1]	0.05 ppm
Meat byproduct (except liver)[1]	0.10 ppm
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Liver[1]	0.20 ppm
Eggs	0.10 ppm

[1] Of cattle, hog, goat, horse and sheep.

Based on the submitted studies, HED also concluded that the currently established tolerances for secondary residues in poultry, meat, fat and meat byproducts should be removed.

HED recommends that conversion of conditional registration to unconditional registration

may be considered upon submission of the following residue chemistry data:

- It has been determined that CGA 205375 should be included in the tolerance expression for livestock commodities. The submitted liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS) method, REM 147.07, will be adequate for enforcement purposes. However, the petitioner should submit a revised method which includes a second MS/MS ion transition as specified by ACB and supply, with replenishment when requested, a standard of metabolite CGA205375 to EPA National Pesticide Standard Repository at ACB laboratory (memo, Charles J. Stafford, ACB Project # B07-26, 10/2907).

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- Two additional tomato (cherry tomato or varieties of tomatoes in which the mature fruits will be less than 2 inches in diameter) field trials should be conducted in Zone 3.

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2.0 PHYSICAL/CHEMICAL PROPERTIES CHARACTERIZATION

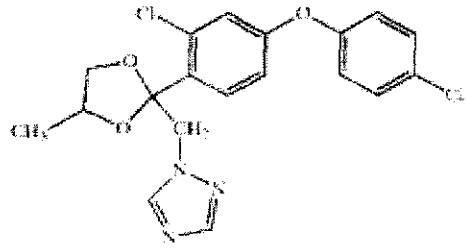
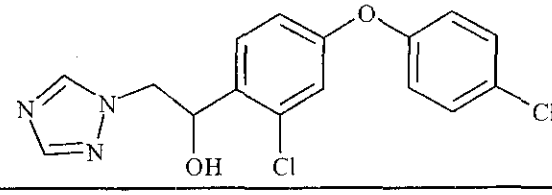
TABLE 2.1. Nomenclature of Difenoconazole and CGA 205375.	
Compound *CGA169374*	Chemical Structure 
Common name	Difenoconazole
Company experimental name	CGA 169374
IUPAC name	*cis-trans*-3-chloro-4-[4-methyl-2-(1*H*-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-2-yl]phenyl 4-chlorophenyl ether
CAS name	1-[2-[2-chloro-4-(4-chlorophenoxy)phenyl]-4-methyl-1,3-dioxolan-2-ylmethyl]-1*H*-1,2,4-triazole
CAS #	119446-68-3
End-use product name	U.S. Registered Products: EPA Reg. Nos. 100-740, 100-814, 100-826, 100-885, 100-935, 100-973, and 100-1141.
Compound	
Common name	CGA-205375
IUPAC name	1-[2-chloro-4-(4-chloro-phenoxy)phenyl]-2-[1,2,4]triazol-1-yl-ethanol

Table 2.2. Physicochemical Properties of Difenoconazole.		
Parameter	Value	Reference
Melting point	78.6 °C	DP#s 172067 and 178394, 10/26/92, R. Lascola
pH	6-8 at 20 °C (saturated solution)	
Density	1.37 g/cm^3 at 20 °C	
Water solubility	3.3 µg/mL at 20 °C	
Solvent solubility	g/100 mL at 25 °C: n-hexane: 0.5 1-octanol: 35 toluene: 77 acetone: 88 ethanol: 89	
Vapor pressure	2.5 x 10^{-10} mmHg at 25 °C	
Dissociation constant, pK_a	<0	
Octanol/water partition coefficient, Log(K_{OW})	4.2 at 25 °C	

Table 2.2. Physicochemical Properties of Difenoconazole.		
Parameter	Value	Reference
UV/visible absorption spectrum	λ_{max} about 200 and 238 nm (in methanol at 26 °C)	PMRA Proposed Regulatory Decision Document (PRDD99-01) on Difenoconazole, 4/14/99

3.0 HAZARD CHARACTERIZATION

A detailed hazard characterization for difenoconazole is presented in HED's previous risk assessment (Memo, S. Levy *et al.*, 23-NOV-1999; DP# 258774). Since the last review, the registrant has submitted new studies which have been reviewed by the agency. The executive summaries for the new studies and updated toxicity profile are provided in the appendix of this risk assessment. The new data did not change significantly the previous health hazard conclusions. In an acute neurotoxicity study in rats, reduced fore-limb grip strength was observed on day 1 in males and clinical sign of neurotoxicity in females at the limit dose of 2000 mg/kg. This effect in males is considered as transient since it was not observed at later observation points and high toxicity in females. In a subchronic neurotoxicity study in rats decreased hind limb strength was observed only in males, which was considered as nonspecific in nature. Therefore, difenoconazole is not considered neurotoxic compound. The newly submitted mutagenicity studies on difenoconazole and its metabolites (CGA 205374 and CGA 205375) were negative for mutagenicity in various metagenic assays. Therefore, it supports the previous conclusion that difenoconazole is not mutagenic. No systemic toxicity was observed at the limit dose in a 28-day dermal toxicity study in rats. A dermal absorption of 15.3% was observed through rat skin using *in vivo* method.

The doses and toxicological endpoints selected for various exposure scenarios applicable to this risk assessment are summarized in Table 3.0.1 and Table 3.0.2.

Table 3.0.1. Summary of Toxicological Doses and Endpoints for Difenoconazole for Use in Dietary and Non-Occupational Human-Health Risk Assessments.

Exposure Scenario	Point of Departure	Uncertainty/FQPA Safety Factors	RfD, PAD, LOC for Risk Assessment	Study and Relevant Toxicological Effects
Acute Dietary (All populations)	NOAEL = 25 mg/kg	UF_A = 10X UF_H = 10X UF_{FQPA} = 1X	aRfD = aPAD = 0.25 mg/kg/day	**Acute Neurotoxicity Study in Rats** LOAEL= 200 mg/kg in males based on reduced fore-limb grip strength in males on day 1.
Chronic Dietary (All populations)	NOAEL = 0.96 mg/kg/day	UF_A = 10X UF_H = 10X UF_{FQPA} = 1X	cRfD = cPAD = 0.01mg/kg/day	**Combined chronic toxicity/carcinogenicity (rat; dietary)** LOAEL = 24.1/32.8 mg/kg/day (M/F) based on cumulative decreases in body-weight gains.
Incidental Oral Short- and Intermediate-Term (1-30 days and 1-6 months)	NOAEL = 1.25 mg/kg/day	UF_A = 10X UF_H = 10X UF_{FQPA} = 1X	Residential LOC for MOE<100	**Reproduction and fertility effects (rat; dietary)** Offspring LOAEL = 12.5 mg/kg/day based on reduction in body-weight of F_1 males.
Dermal Short- and Intermediate-Term (1-30	Oral NOAEL = 1.25 mg/kg/day Dermal	UF_A = 10X UF_H = 10X UF_{FQPA} = 1X	Residential LOC for MOE<100	**Reproduction and fertility effects (rat; dietary)** Offspring LOAEL = 12.5 mg/kg/day based on reduction in body-weight

Table 3.0.1. Summary of Toxicological Doses and Endpoints for Difenoconazole for Use in Dietary and Non-Occupational Human-Health Risk Assessments.

Exposure Scenario	Point of Departure	Uncertainty/FQPA Safety Factors	RfD, PAD, LOC for Risk Assessment	Study and Relevant Toxicological Effects
days and 1-6 months)	Absorption factor=15.3%			gain of F_0 females prior to mating, gestation and lactation.
Dermal Long-Term (>6 months)	Oral NOAEL = 0.96 mg/kg/day Dermal Absorption factor=15.3%	UF_A = 10X UF_H = 10X UF_{FQPA} = 1X	Residential LOC for MOE<100	**Combined chronic toxicity/carcinogenicity (rat; dietary)** LOAEL = 24.1/32.8 mg/kg/day (M/F) based on cumulative decreases in body-weight gains.
Inhalation (Short- and Intermediate-term)	Oral NOAEL = 1.25 mg/kg/day 100% inhalation absorption assumed	UF_A = 10X UF_H = 10X UF_{FQPA} = 1X	Residential LOC for MOE<100	**Reproduction and fertility effects (rat; dietary)** Offspring LOAEL = 12.5 mg/kg/day based on reduction in body weight gain of F_0 females prior to mating, gestation and lactation.
Inhalation (Long- term)	Oral NOAEL = 0.96 mg/kg/day 100% inhalation absorption assumed	UF_A = 10X UF_H = 10X UF_{FQPA} = 1X	Residential LOC for MOE<100	**Combined chronic toxicity/carcinogenicity (rat; dietary)** LOAEL = 24.1/32.8 mg/kg/day (M/F) based on cumulative decreases in body weight gains.
Cancer (oral, dermal, inhalation)	Difenoconazole is classified as a Group C, possible human carcinogen with a non-linear (MOE) approach for human risk characterization (CPRC Document, 7/27/94, Memo, P. V. Shah dated March 3, 2007, HED Doc. No. 0054532).			

Abbreviations: UF = uncertainty factor, UF_A = extrapolation from animal to human (interspecies), UF_H = potential variation in sensitivity among members of the human population (intraspecies), UF_{FQPA} = FQPA Safety Factor, NOAEL = no-observed-adverse-effect level, LOAEL = lowest-observed-adverse-effect level, RfD = reference dose (a = acute, c = chronic), PAD = population-adjusted dose, MOE = margin of exposure, LOC = level of concern.

Table 3.0.2. Summary of Toxicological Doses and Endpoints for Difenoconazole for Use in Occupational Human-Health Risk Assessments.

Exposure Scenario	Point of Departure	Uncertainty/FQPA Safety Factors	RfD, PAD, Level of Concern for Risk Assessment	Study and Toxicological Effects
Dermal Short- and Intermediate-Term (1-30 days and 1-6 months)	Oral NOAEL = 1.25 mg/kg/day Dermal Absorption factor=15.3%	UF_A = 10X UF_H = 10X UF_{FQPA} = 1X	Occupational LOC for MOE<100	**Reproduction and fertility effects (rat; dietary)** Offspring LOAEL = 12.5 mg/kg/day based on reduction in body-weight gain of F_0 females prior to mating, gestation and lactation.
Dermal Long-Term (>6 months)	Oral NOAEL = 0.96 mg/kg/day	UF_A = 10X UF_H = 10X UF_{FQPA} = 1X	Occupational LOC for MOE<100	**Combined chronic toxicity/carcinogenicity (rat; dietary)** LOAEL =

Table 3.0.2. Summary of Toxicological Doses and Endpoints for Difenoconazole for Use in Occupational Human-Health Risk Assessments.

Exposure Scenario	Point of Departure	Uncertainty/FQPA Safety Factors	RfD, PAD, Level of Concern for Risk Assessment	Study and Toxicological Effects
	Dermal Absorption factor=15.3%			24.1/32.8 mg/kg/day (M/F) based on cumulative decreases in body-weight gains.
Inhalation (Short- and Intermediate-term)	Oral NOAEL = 1.25 mg/kg/day 100% inhalation *absorption* assumed	UF_A = 10X UF_H = 10X UF_{FQPA} = 1X	Occupational LOC for MOE<100	**Reproduction and fertility effects (rat; dietary)** Offspring LOAEL = 12.5 mg/kg/day based on reduction in body-weight gain of F_0 females prior to mating, gestation and lactation.
Inhalation (Long- term)	Oral NOAEL = 0.96 mg/kg/day 100% inhalation absorption assumed	UF_A = 10X UF_H = 10X UF_{FQPA} = 1X	Occupational LOC for MOE<100	**Combined chronic toxicity/carcinogenicity (rat; dietary)** LOAEL = 24.1/32.8 mg/kg/day (M/F) based on cumulative decreases in body-weight gains.
Cancer (oral, dermal, inhalation)	Difenoconazole is classified as a Group C, possible human carcinogen with a non-linear (MOE) approach for human risk characterization (CPRC Document, 7/27/94, Memo, P. V. Shah dated March 3, 2007, HED Doc. No. 0054532).			

Abbreviations: *UF = uncertainty factor, UF_A = extrapolation from animal to human (interspecies), UF_H =* potential variation in sensitivity among members of the human population (intraspecies), UF_{FQPA} = FQPA Safety Factor, NOAEL = no-observed-adverse-effect level, LOAEL = lowest-observed-adverse-effect level, RfD = reference dose (a = acute, c = chronic), PAD = population-adjusted dose, MOE = margin of exposure, LOC = level of concern.

3.1 FQPA ASSESSMENT

3.1.1. Determination of Susceptibility

The Hazard Identification Assessment Review Committee (HIARC) determined that the available Agency Guideline studies indicated no increased susceptibility of rats or rabbits to in utero and/or postnatal exposure to difenoconazole. In the prenatal developmental toxicity studies in rats and rabbits and the two-generation reproduction study in rats, toxicity to the fetuses/offspring, when observed, occurred at equivalent or higher doses than in the maternal/parental animals. In the prenatal developmental toxicity study in rats, maternal toxicity was manifested as decreased in body weight gain and food consumption at the LOAEL of 85 mg/kg/day; NOAEL was 16 mg/kg/day. The developmental toxicity was manifested as alterations in fetal ossifications at 171 mg/kg/day; developmental NOAEL was 85 mg/kg/day. In a developmental toxicity study in rabbits, maternal and developmental toxicity were seen at the same dose level (75 mg/kg/day). Maternal toxicity in rabbits were manifested as decreased in body weight gain and decreased in food consumption, while developmental toxicity was manifested as decreased in fetal weight. In a 2-generation reproduction study in rats, decreased in maternal body weight gain and decreased in body weights of F1 males at the LOAEL of 12.5

mg/kg/day; parental systemic and off spring toxicity NOAEL was 1.25 mg/kg/day.

3.1.2. Adequacy of Toxicity Database

There are no data gaps for the assessment of the effects of difenoconazole following in utero and/or postnatal exposure. The acute and subchronic neurotoxicity studies in rats are available. In an acute neurotoxicity study in rats, reduced fore-limb grip strength was observed on day 1 in males. This effect is considered as transient since it was not observed at later observation points. In a subchronic neurotoxicity study in rats decreased hind limb strength was observed only in males, which was considered as nonspecific in nature. There is no evidence of neurotoxicity in the database. EPA concluded that difenoconazole is not a neurotoxic compound. Based on the toxicity profile, and lack neurotoxicity, a developmental neurotoxicity study in rats is not required.

3.1.3. Degree of Concern Analysis:

Since there is no evidence of susceptibility, there is no concern for increased susceptibility due to exposure to difenoconazole.

3.1.4. FQPA Safety Factor Recommendation

The FPQA factor for increased susceptibility to infant and children is reduced to 1x for the following considerations:

1) toxicology data base for difenoconazole is complete;
2) there is no indication of increased susceptibility of rats or rabbit fetuses to in utero and/or postnatal exposure in the developmental and reproductive toxicity data;
3) there are no concerns for neurotoxicity
4) developmental neurotoxicity study is not required
5) The dietary food exposure assessments were performed based on 100% CT and tolerance-level residues.
6) worst-case fate parameters were used in the EFED models for ground and surface source drinking water exposure assessments resulting in estimates that are upper-bound concentrations; and
7) there are currently no registered residential uses for difenoconazole and therefore, exposure to infants and children is not expected.

3.2 Endocrine Disruption

EPA is required under the Federal Food Drug and Cosmetic Act (FFDCA), as amended by FQPA, to develop a screening program to determine whether certain substances (including all pesticide active and other ingredients) "may have an effect in humans that is similar to an effect produced by a naturally occurring estrogen, or other such endocrine effects as the Administrator may designate." Following the recommendations of its Endocrine Disruptor Screening and

Testing Advisory Committee (EDSTAC), EPA determined that there was scientific basis for including, as part of the program, the androgen and thyroid hormone systems, in addition to the estrogen hormone system. EPA also adopted EDSTAC's recommendation that the Program include evaluations of potential effects in wildlife. For pesticide chemicals, EPA will use FIFRA and, to the extent that effects in wildlife may help determine whether a substance may have an effect in humans, FFDCA has authority to require the wildlife evaluations. As the science develops and resources allow, screening of additional hormone systems may be added to the Endocrine Disruptor Screening Program (EDSP).

When the appropriate screening and/or testing protocols being considered under the Agency's EDSP have been developed, difenoconazole may be subjected to additional screening and/or testing to better characterize effects related to endocrine disruption.

4.0 EXPOSURE ASSESSMENT

Residue chemistry summary - Memo, W. Wassell and M. Sahafeyan, 09-AUG-2007; DP#340379
Dietary exposure analysis (difenoconazole) - Memo, M. Sahafeyan, 09-AUG-2007; DP#341303
Dietary exposure analysis (1,2,4-Triazole) - Memo, M. Sahafeyan, 09-AUG-2007; DP#341803
Occupational residential exposure analysis – Memo, M. Dow, 09-AUG-2007; DP#340044
Drinking water summary - EFED Memo, I. Maher, 19-JUN-2007; DP#333319 and 340041

4.1 Summary of Registered Uses

U.S.: In the U.S., difenoconazole is currently registered for seed treatment to control seed-borne and soil-borne diseases of wheat, barley, sweet corn, cotton and canola, and proposed for use (foliar application) on fruiting vegetables, pome fruit, sugar beet, and tuberous and corm vegetables. According to the Agency's Office of Pesticide Program Information Network (OPPIN) database, end-use products containing difenoconazole as the active ingredient are sold in this country under the trade names Dividend®, Dividend® Extreme, Dividend® WS, Dividend® XL, Dividend® XL RTA, Helix®, and Helix® XTRA.

Difenoconazole tolerances have been established in 40 CFR §180.475. Currently, the tolerances for plant and livestock commodities are expressed in terms of difenoconazole *per se*. The tolerance expression is now recommended to include both the parent and the metabolite CGA 205375 for all livestock commodities. Established tolerances for plant commodities range from 0.01 ppm (canola, seed) to 0.2 ppm (imported bananas). Tolerances for milk, eggs, fat, meat, and meat byproducts of cattle, goat, hog, horse, and sheep, and fat, meat, and meat byproducts of poultry currently range from 0.01 to 0.05 ppm.

Import: Difenoconazole is currently registered for seed treatment use of rye and foliar uses on grapes in France, Switzerland, Chile and South Africa. Difenoconazole is also currently registered for foliar uses on pome fruits in Australia, France, New Zealand, South Africa, Switzerland, Chile and Germany. Difenoconazole is now proposed for foliar use registration on papaya in Brazil.

4.2 Summary of Proposed Uses

Syngenta has petitioned the Agency to establish tolerances resulting from foliar uses of difenoconazole on domestic fruiting vegetables, pome fruits, sugar beet, and tuberous and corm vegetables and on imported papaya grown in Brazil.

The petitioner has provided the Agency copies of a domestic label in addition to a foreign label with English translations. A summary of proposed/registered use patterns is listed in Table 4.2.1.

Syngenta has submitted a Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Section 3 request to register an end-use product, Inspire® Fungicide, a 2.08 lb ai/gal emulsifiable concentrate (EC) formulation. The product is proposed for use on fruiting vegetables, sugar beet, and tuberous and corm vegetables as multiple foliar applications at up to 0.11 lb ai/A/application

with a maximum seasonal rate of 0.46 lb ai/A, and proposed for use on pome fruit as multiple foliar applications at up to 0.07 lb ai/A/application with a maximum seasonal rate of 0.33 lb ai/A. The petitioner has proposed preharvest intervals (PHIs) of 0 days for fruiting vegetables, 7 days for sugar beet, and 14 days for pome fruit and tuberous and corm vegetables; a *minimum* retreatment interval (RTI) of 7 days has been proposed for all requested crops. In addition, Syngenta submitted an English translation of a label for a 250 g/L EC formulation, SCORE®, registered for use on papaya grown in Brazil. The product is proposed for use on papaya as 4 foliar applications at up to 0.054 lb ai/A/application (calculated maximum seasonal rate of 0.21 lb ai/A) with 7-10-day RTI.

The submitted labels contain information pertaining to the maximum single application rate, the maximum seasonal rate per growing season, application timing (as it relates to plant growth stage), RTI, application tank-mix preparation, volume of spray mix per unit area, and the PHI. The application rates listed in Table 4.2.1 were copied from the residue chemistry summary document (DP#340379).

Table 4. 2.1. Summary of Directions for Use of Difenoconazole.

Trade Name	Application Timing	Application Rate (lb ai/A)	Max. No. Applic. per Season	RTI[1] (days)	Max. Seasonal Applic. Rate (lb ai/A)	PHI (days)	Use Directions and other Limitations
Fruiting Vegetables [Eggplant; Groundcherry; Pepino; Pepper (includes bell pepper, chili pepper, cooking pepper, pimento, sweet pepper); Tomatillo; Tomato]							
Inspire®	Postemergence	0.07-0.11	Not specified (NS)	7-14	0.46	0	Application to be made in a *minimum* of 15 gal/A using ground equipment or 5 gal/A using aerial equipment. May be used in blocking program using a maximum of two consecutive applications before rotating to fungicides with another mode of action that are registered for the target diseases.
Papaya							
Score®	Postemergence	0.013-0.054 (15-60g ai/ha)	4	7-10	NS 0.21 lb ai/A or 240 g ai/ha implied)	14	Application to begin at the start of fruit formation, at a recommended spray volume of 200-800 L/ha [corresponds to an application rate of 15-60 g ai/ha].
Pome Fruit [Apple; Crabapple; Loquat; Mayhaw; Pear; Pear, Oriental; Quince]							

Table 4. 2.1. Summary of Directions for Use of Difenoconazole.							
Trade Name	Application Timing	Application Rate (lb ai/A)	Max. No. Applic. per Season	RTI[1] (days)	Max. Seasonal Applic. Rate (lb ai/A)	PHI (days)	Use Directions and other Limitations
Inspire®	Postemergence	0.03-0.07	NS	7-14	0.33	14	Application to be made in a minimum of 40 gal/A using ground equipment or 5 gal/A using aerial equipment. To help prevent resistance, it is recommended that no more than 2 consecutive applications with Inspire or another Group 3 fungicide be made before alternating to a different mode of action.
Sugar Beets							
Inspire®	Postemergence	0.07-0.11	NS	7-28	0.46	7	Application to be made in a minimum of 15 gal/A using ground equipment or 5 gal/A using aerial equipment. Application is to be alternated with a non-triazole fungicide that is registered on sugar beets for the target diseases.
Potato and other Tuberous and Corm Vegetables [Arracacha; Arrowroot; Artichoke (Chinese and Jerusalem); Burdock; Canna; Cassava (bitter and sweet); Chayote (root); Chufa; Dasheen (Taro); Ginger; Leren; Tanier; Tumeric; and Yam (bean and true)]							
Inspire®	Postemergence	0.07-0.11	NS	7-14	0.46	14	Application to be made in a minimum of 15 gal/A using ground equipment or 5 gal/A using aerial equipment. May be used in blocking program using a maximum of two consecutive applications before rotating to fungicides with another mode of action that are registered for the target diseases.
Ornamental use (carnation, iris, gladiolus, roses)							
Inspire®	Postemergence	0.03-0.13	4	7-10	0.56	NS	Re-entry interval specified as 12 hours.

Conclusions. The proposed use directions are adequate to allow evaluation of the residue data submitted in support of this petition. Certain amendments to the proposed use directions are

required such that the proposed use directions conform to the use patterns used in the submitted crop field trials:

- The use directions for pome fruit should be modified to state that aerial applications be made in a minimum of 10 gal/A. The guidelines require that aerial applications to orchard crops be made in 10 gal/A, unless data are available to support a lower aerial spray volume; no crop field trial data were submitted to support aerial applications with spray volumes less than 10 gal/A.
- The label for tomato should be revised to include prohibitions against use on fruiting vegetables grown in a greenhouse and on varieties of tomatoes in which the mature tomatoes will be less than 2 inches in diameter (such as cherry tomatoes).
- HED has requested submission of confined rotational crop studies. While these data are being generated the following rotational crop restriction should be added to the label: Rotate only to crops for which difenoconazole is registered.

The label includes instructions for preparing tank mixtures, with a statement that the product is usually compatible with all tank-mix partners listed on the label. However, no tank-mix partners are listed on the label. The instructions for preparing tank mixes should be deleted from the label.

The petitioner may wish to modify the label to specify that applications to fruiting vegetables, pome fruit, sugar beets, and tuberous and corm vegetables may be made with a spray adjuvant, as an adjuvant was used in the submitted crop field trials.

4.3 Dietary Exposure/Risk Pathway

Nature of the Residue - Plants/Livestock: The nature of the residue in plants is understood based on acceptable plant metabolism studies reflecting foliar uses on canola, grape, potato, tomato, and wheat, and seed treatment uses on wheat. HED had previously concluded that the residue of concern for tolerance enforcement and risk assessment is difenoconazole *per se* because of non-foliar use at the time. Because the petitioner has now proposed foliar uses of difenoconazole, which result in higher residues in crop RACs, the need to include metabolite CGA 205375 in the tolerance expression and/or risk assessment has been re-examined. Based upon a review of the previously-submitted metabolism data for difenoconazole, HED concludes the residue of concern for both tolerance setting and risk assessment for the crops included in this petition is difenoconazole *per se*.

The nature of the residue in livestock is adequately understood. Previously submitted ruminant metabolism studies are adequate to support the proposed foliar uses of difenoconazole. HED concludes the residue of concern in all livestock commodities for tolerance setting and risk assessment are difenoconazole and its metabolite CGA 205375.

Residue Analytical Enforcement Methods: An adequate method is available for the enforcement of tolerances for plant commodities, Method AG-575B. This method determines residues of difenoconazole *per se* in crop commodities by gas chromatography (GC) with nitrogen-phosphorus detection (NPD). The limit of quantitation (LOQ) stated in the method is

0.01 ppm for wheat grain and 0.05 ppm for wheat forage and straw. The validated LOQ for crop commodities associated with this petition is 0.01 ppm. Method AG-544A is a GC/NPD method with an LOQ of 0.05 ppm in meat and eggs and 0.01 ppm in milk.

The petitioner has submitted a liquid chromatography (LC)/mass spectrometry (MS)/MS method, REM 147.07, for the determination of residues of difenoconazole and its metabolite CGA 205375 in samples of livestock commodities. Method REM 147.07 was used for data collection in samples from the cattle and hen feeding studies included with the current submission. The method was adequately validated using samples of cattle liver, kidney, muscle, fat, and milk, and eggs. Adequate radiovalidation data were submitted demonstrating that the extraction procedures of method REM 147.07 adequately extract aged residues of difenoconazole, CGA-205375, and 1,2,4-T from hen liver, muscle, fat, and egg yolk. Independent laboratory validation (ILV) data have been submitted for the method using samples of cattle muscle, milk, and egg. Adequate recoveries were obtained from the trialed commodities. HED concludes this method is adequate for tolerance enforcement based on the validation by the Agency (memo, Charles J. Stafford, ACB Project # B07-26, 10/2907) and recoverability of difenoconazole by several existing multiresidue analytical methods used by U.S. Food and Drug Administration (FDA), Department of Agriculture – Pesticide Data Program (USDA – PDP), Germany and Netherland. However, as conditions of registration the petitioner should submit a revised method which includes a second MS/MS ion transition as specified by ACB and supply, with replenishment when requested, a standard of metabolite CGA205375 to EPA National Pesticide Standard Repository at ACB laboratory (memo, Charles J. Stafford, ACB Project # B07-26, 10/2907).

Samples of crop commodities from the submitted crop field trial and processing studies were analyzed for residues of triazole compounds 1,2,4-T, TA, and TAA using an adequate LC/MS/MS method, Method No. Meth-160 (and revisions), and samples of cattle commodities were analyzed for residues of 1,2,4-T using an adequate LC/MS/MS method, RAM 455/01.

Multiresidue Method: Adequate multiresidue method (MRM) data for difenoconazole and metabolite CGA 205375 [1-[2-chloro-4-(4-chloro-phenoxy)-phenyl]-2-[1,2,4]-triazol-1-yl-ethanol] are available. The data indicate that the MRM methods are not useful for the determination of difenoconazole or CGA 205375.

Storage Stability: Samples of raw agricultural and processed commodities from the crop field trial, processing, and field rotational crop studies submitted with this petition were stored frozen for up to ~12 months prior to analysis for residues of difenoconazole. Adequate storage stability data are available indicating that residues of difenoconazole are stable in/on diverse crop RACs (banana, cotton seed, lettuce, potato, soybean seed, tomato, and wheat forage, grain, and straw) during frozen storage for at least 1 year, and that residues are stable in/on processed cotton seed oil and meal during frozen storage for at least 2 years. These data are adequate to support the storage durations and conditions of raw agricultural crop samples from the submitted crop field trial, processing, and field rotational crop studies.

Data were also submitted demonstrating the stability of CGA 205375 in/on apple and grape stored frozen for up to 2 years.

Samples of cattle commodities from the submitted feeding study were stored frozen up to 10 months prior to analysis for residues of difenoconazole and CGA 205375. No supporting storage stability data have been submitted.

RAC samples from the submitted crop field trial and processing studies were stored frozen up to 10 months prior to analysis for 1,2,4-T, TA, and TAA, and samples of cattle commodities were stored frozen for up to 10 months prior to analysis for 1,2,4-T. No storage stability data are available for 1,2,4-T, TA, and TAA.

Meat/Milk/Poultry/Eggs: An adequate cattle feeding study has been submitted, pending submission of supporting storage stability data. The dietary burdens of difenoconazole to beef and dairy cattle have been calculated using the registered and proposed uses. Based on the calculated dietary burdens and the feeding study data, HED concludes that the established tolerances for milk and meat of cattle, goat, hog, horse, and sheep are adequate to support the proposed uses; however, the tolerance expression for all livestock commodities should be changed to include residues of CGA 205375. Additionally, the tolerance levels for residues in meat byproduct (except liver), fat, and liver of cattle, goat, hog, horse, and sheep should be established at 0.10, 0.10 and 0.20 ppm, respectively. The current tolerance levels are 0.05 ppm for residues in fat meat and meat byproduct. For poultry, based on the calculated dietary burdens and the submitted feeding study data, HED concludes that the currently established tolerances for secondary residues in poultry, meat, fat and meat byproducts should be removed. Additionally, the tolerance for residues of difenoconazole in eggs should be altered to include residues of CGA-205375 and the tolerance level should be increased to 0.10 ppm (to account for CGA-205375).

Crop Field Trials: Adequate field trial data are available for papaya, pome fruit (apple and pear), sugar beet, and tuberous and corm vegetables (potato). An adequate number of geographically representative field trials were conducted at 1x the proposed maximum seasonal rate for each crop. The submitted crop field trial data for fruiting vegetables (bell pepper, non-bell pepper, and tomato) are tentatively adequate, pending submission of one additional field trial for cherry tomato and provided the petitioner submits a revised Section B/proposed label. The amended label should include prohibitions against use on fruiting vegetables grown in a greenhouse and on varieties of tomatoes in which the mature tomatoes will be less than 2 inches in diameter (such as cherry tomatoes).

The available field trial data indicate that the proposed tolerances for residues in/on papaya and sugar beet are adequate, but that increased tolerances for residues are needed for the fruiting vegetable group, at 0.60 ppm, and for the pome fruit group, at 1.0 ppm. The proposed tolerance for residues in/on tuberous and corm vegetables is too high; the available data indicate that a tolerance level of 0.01 ppm is appropriate. HED has recently concluded that sugar beet tops are not a significant livestock feed item; therefore, a tolerance for residues in/on sugar beet tops is not required. For each of the crops listed above, the *Guidance for Setting Pesticide Tolerances Based on Field Trial Data* (SOP), along with the tolerance spreadsheet, was used for calculating recommended tolerances.

Processed Food/Feed: Adequate processing data for apple, potato, sugar beet, and tomato are

available. The available data indicate that residues of difenoconazole do not concentrate in apple juice, potato chips, potato granules/flakes, sugar beet molasses, sugar beet refined sugar, or tomato puree; residues were found to concentrate in apple wet pomace, potato wet peel, and sugar beet dried pulp. The processing data indicate that tolerances for residues in/on apple wet pomace, processed potato waste, and sugar beet dried pulp should be proposed at 4.5 ppm, 0.04 ppm, and 1.9 ppm, respectively. Residues of difenoconazole were also found to concentrate in tomato paste; however, expected residues in tomato paste are not substantially greater than the recommended tolerance for the fruiting vegetable group; therefore, a tolerance for tomato paste is not needed.

Confined Accumulation in Rotational Crops: The petitioner has proposed a 60-day plantback interval (PBI) for all crops that do not appear on the product label, with a 30-day PBI for cereals, and root and tuber crops. The available confined rotational crop data are not adequate to support the proposed uses, as the studies were conducted at <0.3x the proposed maximum seasonal rate to annual crops, and there are no data delineating the metabolism of the phenyl portion of the molecule in rotational crops. The petitioner should submit new confined rotational crop studies reflecting application of [^{14}C]difenoconazole, labeled in the phenyl and triazole rings, at 0.46 lb ai/A (1x the proposed maximum seasonal rate to annual crops). The studies should be conducted according to the requirements specified in OPPTS 860.1850. While these data are being generated the following rotational crop restriction must be added to the label: "Rotate only to crops for which difenoconazole is registered."

Tolerance Summary: No maximum residue limits (MRLs) for residues of difenoconazole have been established by Codex Alimentarius. Canadian and Mexican MRLs have been established for difenoconazole; however, no MRLs have been established for the requested crops.

Provided the petitioner submits revised Sections B and F, HED concludes that the residue chemistry database is sufficient for conditional registration and establishment of tolerances for residues of difenoconazole *per se* in/on fruiting vegetables, pome fruit, sugar beets, tuberous and corm vegetables, and imported papaya. Additionally, HED has determined that tolerances should be established for residues of difenoconazole and its metabolite CGA-205375 in/on the milk, meat, meat byproducts, fat, and liver of ruminants. Below in Table 4.3.1 is the proposed and HED-recommended tolerance summary for difenoconazole.

Table 4.3.1 Tolerance Summary for Difenoconazole.

Commodity	Proposed Tolerance (ppm)	Recommended Tolerance (ppm)	Comments; *Changes to Commodity Definition/Tolerance Expression*
Fruit, Pome Group	0.6[1]	1.0	*Fruit, pome, group 11*
Vegetable, Fruiting, Group	0.5	0.60	*Vegetable, fruiting, group 8*
Vegetable, Tuberous and Corm, subgroup	0.02	0.01	*Vegetable, tuberous and corm, subgroup 1C*
Beet, sugar	0.3	0.30	
Beet, sugar, tops	7.0	Remove	Tolerances are not currently required for sugar beet tops.
Papaya	0.3	0.30	
Apple, wet pomace	None proposed	4.5	

Beet, sugar, dried pulp	None proposed	1.9	
Potato, processed waste	None proposed	0.04	
Milk	None proposed	unchanged	Tolerance expression has changed to include difenoconazole and its metabolite CGA 205375.
Meat[2]	None proposed	unchanged	Tolerance expression has changed to include difenoconazole and its metabolite CGA 205375.
Meat byproduct[2] (except liver)	None proposed	0.10	Tolerance expression has changed to include difenoconazole and its metabolite CGA 205375.
Fat[2]	None proposed	0.10	Tolerance expression has changed to include difenoconazole and its metabolite CGA 205375.
Liver	None proposed	0.20	Tolerance expression has changed to include difenoconazole and its metabolite CGA 205375.
Poultry, fat, meat, meat byproducts	0.05	Remove	Tolerances are recommended to be removed.
Eggs	0.05	0.1	Tolerance expression has changed to include difenoconazole and its metabolite CGA 205375.

[1] A tolerance without U.S. registration currently exists for residues of difenoconazole in/on pome fruit at 0.10 ppm.
[2] Of cattle, goat, hog, horse and sheep.

4.4 Water Exposure and Risk Pathway

The following information concerning the environmental fate and drinking water assessment of difenoconazole was provided by EFED (I. Maher, 19-JUN-2007; DP# 333319). At the present time, surface and ground water monitoring data are not available for difenoconazole.

Ground and Surface Water EDWCs: The drinking water residue of concern for risk assessment purposes was decided by the difenoconazole risk assessment team in 2005. Drinking water estimates include surface water EDWCs based on the PRZM/EXAM model (Tier II assessment) and the SCI-GROW groundwater regression model. Both models assumed a treatment rate for difenoconazole on ornamental nurseries at the maximum annual application rate of 0.52 lb ai/A which resulted in the highest estimated water residues among all agricultural and non-agricultural uses.

The EDWC estimates are as follows:

ground water estimate: 0.00128 ppb (acute)
0.00108 ppb (chronic)

surface water estimate: 13.3 ppb; 1-in-10 year annual peak concentration
9.43 ppb; 1-in-10 year average peak concentration

4.5 Dietary-Exposure Analysis

Aggregate (food + water) acute and chronic dietary risk assessments were conducted using DEEM-FCID™ (ver. 2.03) model. This model uses food consumption data from USDA's CSFII; 1994-1996 and 1998. The analyses were performed to support a Section 3 request for new uses of the fungicide difenoconazole in/on fruiting vegetables, pome fruit, sugar beets, tuberous and corm vegetables, and imported papaya.

The Tier 1 acute and chronic analyses assumed tolerance-level residues, 100% CT, and empirical and DEEM™ (ver. 7.76) default processing factors. The resulting acute food exposure estimates were less than HED's level of concern (<100% aPAD) at the 95th percentile of the exposure distribution for the U.S. general population (2.3% aPAD) and all population sub-groups; the most highly exposed population subgroup was all-infants sub-population with 8% aPAD. The resulting chronic food exposure estimates were less than HED's level of concern (<100% cPAD) for U.S. general population (18% cPAD) and all population sub-groups; the most highly exposed population subgroup was children 1-2 years old with 56% cPAD. A cancer dietary assessment was not conducted for difenoconazole because the cancer NOAEL is higher than the chronic RfD; therefore, the chronic dietary risk estimate is more protective.

The acute and chronic aggregate dietary exposure analyses for difenoconazole metabolites 1,2,4-T and TA+TAA from use of all registered and proposed triazole-based pesticides are conducted separately (M. Sahafeyan, DP#341803 and DP#344298) according to the HED recommendation (Memo, M. Doherty, et. al, DP#322215, 7-FEB-2006); see section 6.0 below. The results of acute and chronic aggregate dietary risk assessment for difenoconazole (parent only) are shown in Tables 4.5.1 and 4.5.2 below.

Table 4.5.1. Summary of acute Dietary Exposure and Risk for Difenoconazole at the 95th Percentile.

Population Subgroup	aPAD (mg/kg/day)	Exposure (mg/kg/day)	%cPAD
General U.S. Population	0.25	0.005772	2
All Infants (< 1 year old)		**0.020281**	**8**
Children 1-2 years old		0.016442	7
Children 3-5 years old		0.013839	6
Children 6-12 years old		0.008167	3
Youth 13-19 years old		0.003944	2
Females 13-49 years old		0.003842	2
Adults 20-49 years old		0.004267	2
Adults 50+ years old		0.003882	2

Table 4.5.2. Summary of Chronic Dietary Exposure and Risk for Difenoconazole.			
Population Subgroup	**cPAD (mg/kg/day)**	**Exposure (mg/kg/day)**	**%cPAD**
General U.S. Population	0.01	0.001828	18
All Infants (< 1 year old)		0.005058	51
Children 1-2 years old		**0.005579**	**56**
Children 3-5 years old		0.004575	46
Children 6-12 years old		0.002670	27
Youth 13-19 years old		0.001307	13
Females 13-49 years old		0.001292	13
Adults 20-49 years old		0.001320	13
Adults 50+ years old		0.001433	14

The bolded %cPAD is the highest.

4.6 Residential Exposure and Risk Pathway

HED believes residential pesticide handlers will be exposed to short-term duration (1 - 30 days) only.

The dermal and inhalation (short-term) residential exposure was assessed for "homeowners" mixer/loader/applicator wearing short pants and short-sleeved shirts as well as shoes plus socks using garden hose-end sprayer, "pump-up" compressed air sprayer, and backpack sprayer. A MOE of 100 is adequate to protect residential pesticide handlers from exposures to difenoconazole. MOEs are >100; therefore are not of concern.

Table 4.6.1 Summary of Exposure & Risk for Homeowners Applying Difenoconazole.				
Unit Exposure[1] mg ai/lb handled	Applic. Rate[2] lb ai/unit	Units Treated[3]	Avg. Daily Exposure[4] mg ai/kg bw/day	Short-term MOE[5]
Mixer/Loader/Applicator Using Garden Hose-end Sprayer				
Dermal: SS&SP 11 Inhal. 0.017	0.13 lb ai/A	0.5 A/day	Dermal: shrtsl&pants 0.00156 Inhal. 0.0000158	790
Mixer/Loader/Applicator Using "Pump-Up" Compressed Air Sprayer				
Dermal: SS&SP 38 Inhal 0.0027	0.13 lb ai/A	0.5 A/day	Dermal: shrtsl&pants 0.00539 Inhal. 0.0000025	230
Mixer/Loader/Applicator Using Backpack Sprayer				
Dermal: SS&SP 5.1 Inhal. 0.03	0.13 lb ai/A	0.5 A/day	Dermal: shrtslv&pants 0.000725 Inhal. 0.000028	1,700

1. Unit Exposures are taken from "PHED SURROGATE EXPOSURE GUIDE", Estimates of Worker Exposure from The Pesticide Handler

Exposure Database Version 1.1, August 1998. Inhal. = Inhalation. Units = mg a.i./pound of active ingredient handled. Unit exposures are also taken from ORETF studies OMA 004,OMA006 and from the Draft Residential SOPs, DECEMBER 1997. SS & SP = short sleeved shirt and short pants. LS & LP = long sleeved shirt and long pants.

2. Applic. Rate. = Taken from the draft Inspire® label.
3. Units Treated are taken the residential SOPs.
4. Average Daily Dose (ADD) = Unit Exposure * Applic. Rate * Units Treated * absorption factor (15.3 % for dermal) ÷ Body Weight (70 kg).
5. NOAEL = No Observable Adverse Effect Level (1.25 mg a.i./kg bw/day for short-term and intermediate-term dermal and inhalation).
6. MOE = Margin of Exposure = No Observable Adverse Effect Level (NOAEL) ÷ ADD. ADD = dermal + inhalation.

With respect to residential post-application exposures, current HED policy (see ExpoSAC minutes from 8/19/99 and 10/11/01) specifies that no significant post-application exposure is anticipated from ornamentals, either by residents or professional applicators; therefore, no residential post-application assessment was conducted.

5.0 AGGREGATE RISK ASSESSMENTS AND RISK CHARACTERIZATION

Including all existing and proposed uses, human-health risk assessments have been conducted for the following exposure scenarios: chronic dietary exposures (food + water) + residential short-term exposure (dermal + inhalation). **The aggregate exposure and risk estimates are not of concern.**

Short-Term Aggregate Risk Assessment: Since a common endpoint has been identified for assessment of short-term oral, dermal, and inhalation exposures (changes in body weights and body-weight gains) the short-term aggregate risk assessment considered exposure from food, water, and residential sources. Since the doses corresponding to the identified oral, dermal, and inhalation endpoints were different but the level of concern for all three routes of exposure are identical, the short-term aggregate exposures were calculated using the 1÷MOE approach. HED combines chronic dietary (food and water) exposure estimates with residential exposure estimates when conducting short-term aggregate risk assessments. Short-term exposure has been defined as from 1- 30 days and HED has concluded that chronic dietary exposure estimates will more accurately reflect actual dietary exposure over these time periods than will high-end acute-dietary exposures. The proposed residential scenarios result in exposure to only adults. Therefore, short-term aggregate assessments were not conducted for infants and children. Table 5.0.1 is a summary of the short-term aggregate exposures and risk estimates. Since the aggregate MOEs are ≥170, short-term aggregate exposure to difenoconazole is not of concern.

Table 5.0.1. Short-Term Aggregate Exposure

Population	Target Aggregate MOE[1]	dietary MOE[2]	dermal + inhalation MOE[3]	agg. MOE (dietary and residential)[4]
Youth 13-19 years old	*100*	730	*230*	180
Adults 20-49 years old		730		180
Adults 50+ years old		670		170
Females 13-49 years old		740		180

[1] total uncertainty factor for all routes of exposure is 100x; therefore, the target MOE is 100.

[2] dietary MOE = short-term incidental oral NOAEL ÷ chronic dietary exposure.

[3] dermal MOE = short-term dermal NOAEL ÷(dermal + inhalation residential exposure) (see text).

[4] aggregate MOE (dietary and residential) = $1 \div ((1 \div MOE_{dietary}) + (1 \div MOE_{dermal}) + (1 \div MOE_{inhalation}))$.

6.0 CUMULATIVE RISK

The Agency did not perform a cumulative risk assessment as part of this tolerance action for difenoconazole. However, the Agency does have concern about potential toxicity to 1,2,4-T and two conjugates, TA and TAA, metabolites common to most of the triazole fungicides. The acute and chronic aggregate (food + water) dietary exposure analyses for 1,2,4-T, and TA+TAA from use of all registered and proposed triazole-based pesticides were updated in separate memorandums (M. Sahafeyan, DP#341803 and DP#344298) according to the HED recommendation (Memo, M. Doherty, *et al*, DP#322215, 7-FEB-2006) to include the new difenoconazole proposed uses. These analyses indicate that the acute and chronic risk from dietary exposure to 1,2,4-T and TA+TAA from all registered and proposed triazole-based pesticides are not of concern.

The results of acute and chronic aggregate dietary exposure analysis for 1,2,4-T indicate that the highest aPAD is 32% for the all-infants population sub-group at the 95%ile of exposure distribution and the highest cPAD is 41% for children 1-2 years old, both below HEDs level of concern(<100% aPAD and <100% cPAD).

The results of acute and chronic aggregate dietary exposure analysis for TA+TAA indicate that the aPAD for females 13-49 years old (the only group with toxicological end point) is 28% at the 95th percentile of exposure distribution and the highest cPAD is 27% for children 1-2 years old, both below HEDs level of concern(<100% aPAD and <100% cPAD).

The new proposed use of difenoconazole on ornamental plants, does not warrant a new cumulative aggregate risk (dietary + residential) for 1,2,4-T. This is because, in the previous cumulative aggregate risk assessment (Memo, M. Doherty, DP#322238, 1-NOV-2005), triadimefon, a triazole-based pesticide, with potentially much higher exposures to residential handlers than difenoconazole were used and the risks were of no concern to HED; therefore, 1,2,4-T aggregate risk due to the addition of ornamental use of difenoconazole are not of concern. For triazole conjugates (TA and TAA), HED does not expect residues of TA and TAA on leaf surfaces due to the formation of TA and TAA from 1,2,4-T within plants; therefore, HED has not conducted a residential exposure assessment for the triazole conjugates.

7.0 OCCUPATIONAL EXPOSURE

7.1 Handler Exposure

Based upon the proposed use patterns, HED believes the most highly exposed occupational pesticide handlers will be mixer/loaders using open pour loading of liquids and applicators using ground-boom spray machinery, airblast sprayer, fixed-wing aircraft, garden hose-end sprayer, "pump-up" (compressed air) sprayer, backpack sprayer and high pressure hand-wand sprayer. HED believes occupational pesticide handlers will be exposed to short-term duration (1 - 30 days) exposures, but not to intermediate-term (1 - 6 months) duration exposures. Although multiple applications are possible, they are separated by 10 - 14 day retreatment intervals. No more than 2 consecutive applications should be made. It is unlikely that pesticide handlers would be exposed continuously for 30 days or more. However, because the short-term and intermediate-term toxicological endpoints are the same, the assessment of short-term exposure and risk is adequate to describe risk from an intermediate-term exposure, should that occur.

Exposures and risks were estimated for occupational handlers applying difenoconazole. Included in Table 7.1.1 are the following occupational pesticide handler scenarios:

1) Mixer/loaders using open pour loading of liquids (PHED);
2) Applicator using open-cab ground-boom sprayer (PHED);
3) Applicator using open-cab airblast sprayer (PHED);
4) Aerial applicator (PHED)
5) Mixer/Loader/Applicator using "pump-up" compressed air sprayer (ORETF);
6) Mixer/Loader/Applicator using backpack sprayer (PHED); and
7) Applicator using high-pressure hand-wand sprayer (PHED).

A MOE of 100 is adequate to protect occupational pesticide handlers from exposures to difenoconazole. Provided occupational handlers wear protective gloves, all MOEs are > 100; therefore, these estimated exposures are not of concern.

Table 7.1.1. Summary of Exposure & Risk for Occupational Handlers Applying Difenoconazole.

Unit Exposure[1] mg ai/lb handled	Applic. Rate[2] lb ai/unit	Units Treated[3]	Avg. Daily Exposure[4] mg ai/kg bw/day	Short-term MOE[5]
Mixer/Loader Using Open Pour Loading of Liquids				
Dermal: SLNoGlove 2.9 HC SLWithGlove 0.023 HC Inhal. 0.0012 HC	0.11 lb ai/A	350 A/day	Dermal: SLNoGlove 0.244 SLWithGlove 0.0019 Inhal. 0.00066	 5 488
Applicator Using Open-Cab Ground-boom Sprayer				
Dermal: SLNoGlove 0.014 HC SLWithGlove 0.014 MC Inhal. 0.00074 HC	0.11 lb ai/A	80 A/day	Dermal: SLNoGlove 0.000269 SLWithGlove 0.000269 Inhal. 0.000093	 3,453 3,453
Applicator Using Open-cab Airblast Sprayer				
Dermal:	0.07 lb ai/A	40 A/day	Dermal:	

Table 7.1.1. Summary of Exposure & Risk for Occupational Handlers Applying Difenoconazole.				
SLNoGlove 0.36 HC SLWithGlove 0.24 HC Inhal. 0.0045 HC			SLNoGlove 0.0022 SLWithGlove 0.00147 Inhal. 0.00018	525 758
Aerial Applicator (Pilots not required to wear gloves)				
Dermal: SLNoGlove 0.0050 MC Inhal. 0.000068 MC	0.11 lb ai/A	350 A/day	Dermal: SLNoGlove 0.00042 Inhal. 0.000037	2735
Mixer/Loader/Applicator Using "Pump-Up" Compressed Air Sprayer				
Dermal: SLWithGlove 0.33 **LCO** Inhal 0.0027	0.13 lb ai/A	5.0 A/day	Dermal: SLWithGlove 0.000469 Inhal. 0.000025	2,530
Mixer/Loader/Applicator Using Backpack Sprayer				
Dermal: SLWithGlove 2.5 **LCO** LC Inhal. 0.03 LC	0.13 lb ai/A	5.0 A/day	Dermal: SLWithGlove 0.00355 Inhal 0.00028	326
Applicator Using High-Pressure Handwand Sprayer				
Dermal: SLWithGlove 0.64 **LCO** LC Inhal. 0.079 LC	0.13 lb ai/A	5.0 A/day	Dermal: SLWithGlove 0.00091 Inhal. 0.000734	760

1. Unit Exposures are taken from "PHED SURROGATE EXPOSURE GUIDE", Estimates of Worker Exposure from The Pesticide Handler Exposure Database Version 1.1, August 1998. Inhal. = Inhalation. Units = mg a.i./pound of active ingredient handled. Data Confidence: LC = Low Confidence, MC = Medium Confidence, HC = High Confidence. Unit exposures are also taken from ORETF studies OMA 004,OMA006 and from the Draft Residential SOPs, DECEMBER 1997. SLNoGlove = single layer of work clothing (long pants, long-sleeved shirt, shoes plus socks) and NO protective gloves. SLWithGloves = single layer work clothing AND WITH the use of protective gloves.
2. Applic. Rate. = Taken from the draft Inspire® label.
3. Units Treated are taken the residential SOPs
4. Average Daily Dose (ADD) = Unit Exposure * Applic. Rate * Units Treated * absorption factor (15.3 % for dermal) ÷ Body Weight (70 kg).
5. NOAEL = No Observable Adverse Effect Level (1.25 mg a.i./kg bw/day for short-term and intermediate-term dermal and inhalation)
6. MOE = Margin of Exposure = No Observable Adverse Effect Level (NOAEL) ÷ ADD. ADD = dermal + inhalation.

7.2 Post-Application Worker Exposure

It is possible for agricultural workers to have post-application exposures to pesticide residues during the course of typical agricultural activities. HED in conjunction with the Agricultural Re-entry Task Force (ARTF) has identified a number of post-application agricultural activities that may occur and which may result in post-application exposures to pesticide residues. HED estimated the post-application exposure for two uses that are considered to have the highest Transfer Coefficients (TC): floricultural crops with a conservative TC of 5,000 cm^2/hr for hand harvesting, pinching and thinning and pome fruits with TC of 3,000 cm^2/hr for thinning.

Floricultural post-application activities

0.13 lb a.i./A * 0.20 * $(1-0)^0$ * 4.54 x 10^8 µg/lb * 2.47 x10^{-8} A/cm^2 = 0.29 µg/cm^2; therefore,

0.29 µg/cm^2 * 5,000 cm^2/hr * 8 hr/day * 0.001 mg/µg * 0.153 (15.3 % dermal absorption) ÷ 70 kg bw = 0.025 mg/kg bw/day.

MOE = NOAEL ÷ ADD then 1.25 mg/kg bw/day ÷ 0.025 mg/kg bw/day = **50.**

A MOE of 100 is adequate to protect agricultural workers from post-application exposures. The

short-term duration MOE is <100, and therefore, is of concern. Post-application activities with a TC >2,500 cm^2/hr will result in MOEs of concern.

HED assumes post-application dislodgeable foliar residue dissipates at a rate of 10% per day. At that rate of dissipation, for floricultural activities with an application rate of 0.13 lb ai/A and a TC of 5,000 cm^2/hr, it is post-application day 10 before MOEs of 100 are attained. For floricultural activities, under these circumstances a restricted entry interval of 10 days is necessary to protect agricultural workers. Use of the lower rate of application (0.03 lb ai/A), results in MOEs that are not of concern.

Pome fruit post-application activities

For the proposed crop use sites, the crop with the next highest TC is pome fruit with a TC of 3,000 cm^2/hr for hand thinning. However, it should be noted that the rate of application to pome fruit is 0.07 lb ai/A as opposed to 0.13 lb ai/A for the ornamentals uses. Therefore:

0.07 lb a.i./A * 0.20 * $(1\text{-}0)^0$ * 4.54 x 10^8 µg/lb * 2.47 x10^{-8} A/cm^2 = 0.157 µg/cm^2 , therefore,

0.157 µg/cm^2 * 3,000 cm^2/hr * 8 hr/day * 0.001 mg/µg * 0.153 (15.3 % dermal absorption) ÷ 70 kg bw = 0.0082 mg/kg bw/day.

MOE = NOAEL ÷ ADD then 1.25 mg/kg bw/day ÷ 0.0082 mg/kg bw/day = **150.**

Since the MOE is >100, the proposed use does not result in a risk of concern under these application parameters.

7.3 Restricted Entry Interval (REI)

Difenoconazole is classified in acute toxicity category III for acute dermal toxicity and primary eye irritation. It is classified in Toxicity Category IV for acute inhalation toxicity and primary skin irritation. It is negative as a dermal sensitizer. Therefore, the interim worker protection standard (WPS) REI of 12 hours is adequate to protect agricultural workers from a most of post-application exposures to difenoconazole. The draft Inspire® label lists the REI as 12 hours. **Post-application exposure to floricultural crops treated at the highest rate of application results in residues of concern, thus an extended REI (10 days) is necessary for that use pattern.**

8.0 DEFICIENCIES/DATA NEEDS

8.1 Toxicology

- None

8.2 Chemistry

860.1200 Directions for Use

- The use directions for pome fruit should be modified to state that aerial applications be made in a minimum of 10 gal/A; no crop field trial data were submitted to support aerial applications with spray volumes less than 10 gal/A.

- Because no crop field trial data were submitted for greenhouse-grown fruiting vegetables, the proposed use directions must be modified to specify that application may not be made to fruiting vegetables grown in a greenhouse.

- As the submitted crop field trial data did not include any small varieties of tomato. While the data for small diameter tomatoes are being generated, a revised Section B/label is required to include the statement "Do not use on varieties in which the mature tomatoes will be less than 2 inches in diameter (such as cherry tomatoes)." This label restriction may be dropped upon receipt and evaluation of the field trial data on cherry tomatoes.

- The label includes instructions for preparing tank mixtures, with a statement that the product is usually compatible with all tank-mix partners listed on the label. However, no tank-mix partners are listed on the label. The instructions for preparing tank mixes should be deleted from the label.

- HED has requested submission of confined rotational crop studies. While these data are being generated the following rotational crop restriction must be added to the label: Rotate only to crops for which difenoconazole is registered.

860.1340 Residue Analytical Methods - Livestock Commodities

- It has been determined that CGA 205375 should be included in the tolerance expression for livestock commodities. The submitted liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS) method, REM 147.07, is adequate for enforcement purposes based on validation by the Agency (Memo, Charles J. Stafford, ACB Project # B07-26, 10/29/07).

- Confirmatory analysis procedures (a second MS/MS ion transition) are needed for REM 147.07 for both parent and the metabolite as specified by ACB (memo, Chales J. Staffor, ACB Project # B07-26, 10/2907).

- Analytical standards for the metabolites of metabolite CGA are currently not available in the National Pesticide Standards Repository [Source: memo, Chales J. Staffor, ACB Project # B07-26, 10/2907]. Analytical reference standards of metabolite CGA should be supplied and supplies replenished as requested by the Repository.

- The petitioner should revise the method to correct all references to "crop matrices" as the method is intended for livestock commodities.

860.1380 Storage Stability

- Data must be submitted depicting the stability of residues of difenoconazole and CGA 205375 in milk and cattle tissues during frozen storage for up to 10 months for milk and 9 months for tissues. The studies cited by the petitioner (report numbers ABR-93012 and 202/99), which contain storage stability data for difenoconazole and CGA 205375, should be submitted.

- Data must be submitted depicting the stability of residues of difenoconazole and CGA 205375 in egg and poultry tissue samples during frozen storage for up to 7 months for egg and 6 months for tissue samples. The studies cited by the petitioner (report numbers ABR-93012 and 202/99), which contain storage stability data for difenoconazole and CGA 205375, should be submitted.

- Storage stability data for residues of 1,2,4-T, TA, and TAA have not been submitted in conjunction with the subject petitions. However, storage stability data for these compounds has been requested as part of the Human-Health Aggregate Risk Assessment for 1,2,4-T, TA and TAA (M. Doherty, *et. al.* 2/7/06). Submission of the data requested in the 2/7/06 document will satisfy storage stability data requirement for the subject petitions.

860.1500 Crop Field Trials

- The submitted residue data for fruiting vegetables are not adequate to fulfill data requirements as an insufficient number of crop field trials was conducted for tomatoes; two additional trials must be conducted in Zone 3. The submitted crop field trial data did not include any small varieties of tomato; therefore, the requested trials should be conducted on a cherry tomato or varieties of tomatoes in which the mature fruits will be less than 2 inches in diameter.

860.1850 Confined Accumulation in Rotational Crops

- The available confined rotational crop data are not adequate to support the proposed uses, as the studies were conducted at <0.3x the proposed maximum seasonal rate to annual crops, and there are no data delineating the metabolism of the phenyl portion of the molecule in rotational crops. The petitioner should submit new confined rotational crop studies reflecting application of [^{14}C]difenoconazole, labeled in the phenyl and triazole

rings, at 0.46 lb ai/A (1x the proposed maximum seasonal rate to annual crops). The studies should be conducted according to the requirements specified in OPPTS 860.1850.

860.1550 Proposed Tolerances

- The proposed tolerances should be revised to reflect the recommended tolerance levels and correct commodity definitions as specified in Table 4.3.1.

8.3 Occupational/Residential

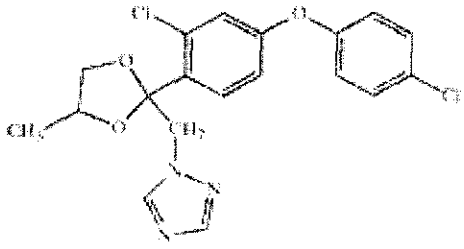
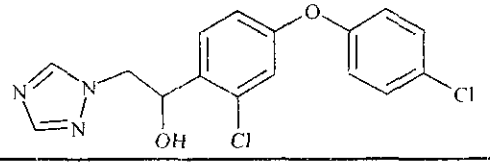
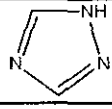
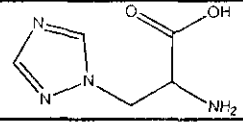
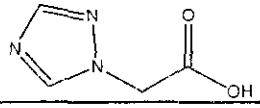
- REI should be extended to 10 days to protect agricultural workers for floricultural activities or maximum application rate should be lowered to 0.03 lb ai/A for that use pattern.

cc: M. Sahafeyan (RAB1), W. B. Greear (RAB1), W.D. Wassell (RAB1)
RDI: RAB1 Branch (8-AUG-2007), G.F. Kramer (9-NOV-2007), RAB1 Chemists (9-NOV-2007)
M. Sahafeyan:S10944:PY1:(703)-305-0776
Petition Number: PP#6F7115
DP#: 346591, 340379, 341303, 341803, and 340044
PC Code: 128847

Attachments:

Attachment 1- Chemical Structures
Attachment 2- Toxicity Profile

Attachment 1: Chemical Structures

TABLE A.1. Compounds Nomenclature.	
Compound	Chemical Structure 
Common name	Difenoconazole
Company experimental name	CGA 169374
IUPAC name	*cis-trans*-3-chloro-4-[4-methyl-2-(1*H*-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-2-yl]phenyl 4-chlorophenyl ether
CAS name	1-[2-[2-chloro-4-(4-chlorophenoxy)phenyl]-4-methyl-1,3-dioxolan-2-ylmethyl]-1*H*-1,2,4-triazole
Compound	
Common name	CGA-205375
IUPAC name	1-[2-chloro-4-(4-chloro-phenoxy)phenyl]-2-[1,2,4]triazol-1-yl-ethanol
Compound	
Common name	1,2,4-Triazole (T)
Company experimental name	CGA071019
IUPAC name	4*H*-1,2,4-Triazole
CAS name	1*H*-1,2,4-Triazole
CAS #	288-88-0
Compound	
Common name	Triazole Alanine (TA)
Company experimental name	CGA131013
IUPAC name	2-Amino-3-1,2,4-triazole-1-yl-propionic acid
CAS name	1*H*-1,2,4-Triazole-1-propanioic acid, α-amino-
CAS #	86362-20-1
Compound	
Common name	Triazole Acetic Acid (TAA)
Company experimental name	CGA142856
IUPAC name	1,2,4-Triazol-1-yl-acetic acid
CAS name	1*H*-1,2,4-Triazole-1-acetic acid
CAS #	28711-29-7

Attachment 2: Toxicity Profiles

Table A.1.1 Acute Toxicity Profile - Difenoconazole

Guideline No.	Study Type	MRID(s)	Results	Toxicity Category
870.1100	Acute oral rat	42090006	LD_{50} = 1450 mg/kg	III
870.1200	Acute dermal rat	42090007	LD_{50} > 2010 mg/kg	III
870.1300	Acute inhalation rat	42090008	LC_{50} > 3.3 mg/L	III
870.2400	Acute eye irritation rabbit	42090009	Mild ocular irritation reversible in 7 days	III
870.2500	Acute dermal irritation rabbit	42090010	Slight irritation	IV
870.2600	Skin sensitization mouse	42090011, 42710004	Negative	N/A

Table A.1.2 Subchronic, Chronic and Other Toxicity Profile of Difenoconazole

Guideline No.	Study Type	MRID No. (year)/ Classification /Doses	Results
870.3100	90-Day oral toxicity (rat)	42090022 (1987) Acceptable/guideline 0, 20, 200, 750, 1500 or 3000 ppm 0, 1, 10, 37.5, 75 and 150 mg/kg/d	NOAEL = 20 ppm (1 mg/kg/day) LOAEL = 200 ppm (10 mg/kg/day) based on the 10% decrease in body weight in the 200 ppm females (as well as a negative trend in feed consumption) and Increases in absolute liver weights in both sexes
870.3100	90-Day oral toxicity (mouse)	42090021 (1987) Minimum/guideline 0, 20, 200, 2500, 7500 or 15,000 ppm M: 0, 2.9, 30.8, 383.6, 1125 and 2250 mg/kg/d F: 0, 4.1, 41.5, 558.9, 1125 and 2250 mg/kg/d	NOAEL = 20 ppm (2.9 mg/kg/day) LOAEL = 200 ppm (30.8 mg/kg/day) based on body weight changes & liver histopathology.
870.3150	26-Week oral toxicity	42090012 (1987) Minimum/ guideline 0, 100, 1000, 3000 or 6000 ppm M: 0, 3.6, 31.3, 96.6 and 157.8 mg/kg/d F: 0, 3.4, 34.8, 110.6 and 203.7 mg/kg/d	NOAEL = 3000 ppm (31.3 mg/kg/day in males/34.8 mg/kg/day in females) LOAEL = 6000 ppm (96.6 mg/kg/day in males/110.6 mg/kg/day in females), based primarily on microscopic examination of CGA 169374-related lenticular cataracts.
870.3200	21/28-Day dermal toxicity (rat)	42090013 (1987) Minimum/ guideline 0, 10, 100 and 1000 mg/kg/d	NOAEL = 10 mg/kg/day LOAEL = 100 mg/kg/day based on statistically significant decrements in body weight, body weight gain, and food consumption.
870.3200	21/28-Day dermal toxicity (rat)	46950310 (2000) Acceptable/ guideline 0, 10, 100 and 1000 mg/kg/d	NOAEL (systemic) = 1000 mg/kg/day LOAEL (systemic) was not determined. NOAEL (dermal) = 100 mg/kg/day LOAEL (dermal) = 1000 mg/kg/day based on hyperkeratosis at the skin application site.
870.3700a	Prenatal developmental in (rat)	42090016, 42710007 (1987) Minimum/ guideline 0, 2, 20, 100 or 200 mg/kg/d from GD 6-15 (nominal doses differed widely from theoretical, this required altering NOAEL/LOAEL values)	**Maternal** NOAEL = 16 mg/kg/day LOAEL = 85 mg/kg/day based on decreased body weight gain and food consumption. **Developmental** NOAEL = 85 mg/kg/day LOAEL = 171 mg/kg/day based on alterations in fetal ossification.

Table A.1.2 Subchronic, Chronic and Other Toxicity Profile of Difenoconazole

Guideline No.	Study Type	MRID No. (year)/ Classification /Doses	Results
870.3700b	Prenatal developmental in (rabbit)	42090017, 42710008 (1987) Minimum/ guideline 0, 1, 25 or 75 mg/kg/d from GD 7-19	**Maternal** NOAEL = 25 mg/kg/day LOAEL = 75 mg/kg/day based on decreased body weight gain and food consumption. **Developmental** NOAEL = 25 mg/kg/day LOAEL = 75 mg/kg/day based on nonsignificant increases in postimplantation loss and resorptions/doe and a significant decrease in fetal weight.
870.3800	Reproduction and fertility effects (rat)	42090018 (1988) Minimum/ guideline 0, 25, 250 or 2500 ppm 0, 1.25, 12.5 and 125 mg/kg/d	**Parental/Systemic** NOAEL = 25 ppm (1.25 mg/kg/day) LOAEL = 250 ppm (12.5 mg/kg/day) based on reductions (statistically nonsignificant) in body weight gain which appear to be part of a dose-related trend days 70-77 prior to mating, days 0-7 of gestation, and days 7-14 of lactation **Reproductive** NOAEL = 25 ppm (1.25 mg/kg/day) LOAEL = 250 ppm (12.5 mg/kg/day) based on a significant reduction in the body weight of F1 male pups at day 21 in the 250 ppm group.
870.4100b	Chronic toxicity (dog)	42090012, 42710005 (1988) Minimum/ guideline 0, 20, 100, 500 or 1500 ppm M: 0, 0.71, 3.4, 16.4 and 51.2 mg/kg/d F: 0, 0.63, 3.7, 19.4 and 44.3 mg/kg/d	NOAEL = 100 ppm (3.4 mg/kg/day in males/3.7 mg/kg/day in females) LOAEL = 500 ppm (16.4 mg/kg/day in males/19.4 mg/kg/day in females), based on significant inhibition of body weight gain in females.
870.4200	Carcinogenicity (rat)	42090019, 42710010 (1989) Minimum/ guideline 0, 10, 20, 500 or 2500 ppm M: o, 0.48, 0.96, 24.12 and 123.7 mg/kg/d F: 0, 0.64, 1.27, 32.79 and 169.6 mg/kg/d	NOAEL = 20 ppm (0.96 mg/kg/day in males/1.27 mg/kg/day in females) LOAEL = 500 ppm (24.1 mg/kg/day in males/ 32.8 mg/kg/day in females) based on reductions in cumulative body weight gains in the 500 and 2500 ppm groups. **No evidence of carcinogenicity**

Table A.1.2 Subchronic, Chronic and Other Toxicity Profile of Difenoconazole

Guideline No.	Study Type	MRID No. (year)/ Classification /Doses	Results
870.4300	Carcinogenicity (mouse)	42090015, 42710006 (1989) Minimum/ guideline 0, 10, 30, 300, 2500 or 3000 ppm M: 0, 1.51, 4.65, 46.29, 423.1 and 818.9 mg/kg/d F: 0, 1.9, 5.63, 57.79 and 512.6 mg/kg/d	NOAEL = 30 ppm (4.7 mg/kg/day in males/5.6 mg/kg/day in females) LOAEL = 300 ppm (46.3 mg/kg/day in males/57.8 mg/kg/day in females) based on reductions in the cumulative body weight gains in the 300, 2500 & 4500 ppm groups. **Evidence of carcinogenicity (liver adenoma/carcinoma in both sexes)**
870.5100	*In vitro* bacterial gene mutation *(Salmonella typhimurium/ E. coli)*/ mammalian activation gene mutation assay	42090019, 42710010 (1989) Minimum/ guideline 340 - 5447 µg/plate; 85 - 1362 µg/plate (repeat assay with TA1537 and TA98)	There were sufficient and valid data to conclude that CGA 169374 technical was negative in the microbial gene mutation assay.
870.5300	*in vitro* mammalian cell gene mutation assay in mouse lymphoma cells	42090024 (1986) Unacceptable/ guideline	No conclusion can be reached from the three nonactivated and two S9 activated mouse lymphoma forward mutation assays conducted with difenoconazole technical. The study was seriously compromised.
870.5375	*In vitro* Mammalian Cytogenetics (chromosomal aberrations) assay in Chinese hamster *CHO cells*	46950319 (2001) Acceptable/ guideline 0, 21.99, 27.49, or 34.36 µg/mL (-S9) 0, 34.36, 53.69 or 67.11 µg/mL (+S9)	There was evidence of a weak induction of structural chromosomal aberrations over background in the presence of S9-mix.
870.5375	*In vitro* Mammalian Cytogenetics (chromosomal aberrations) assay in Chinese hamster CHO cells	46950321 (2001) Acceptable/ guideline 0, 26.3, 39.5 or 59.3 µg/mL (-S9) 0, 11.7 or 17.6 µg/mL (+S9)	There was evidence of a weak induction of structural chromosomal aberrations over background.

Table A.1.2 Subchronic, Chronic and Other Toxicity Profile of Difenoconazole

Guideline No.	Study Type	MRID No. (year)/ Classification /Doses	Results
870.5375	*In vitro* Mammalian Cytogenetics (chromosomal aberrations) assay in human lymphocytes	46950323 (2001) Acceptable/ guideline 0, 5, 30 or 75 µg/mL (-S9) 0, 5, 30 or 62 µg/mL (+S9)	There was no evidence of structural chromosomal aberrations induced over background.
870.5385	*In vivo* mammalian chromosomal aberration test Assay in Mice	42090023 (1986) Unacceptable/guideline 250, 500 or 1000 mg/kg	There was no evidence of a cytotoxic effect on the target organ or significant increase in the frequency of nuclear anomalies (micronuclei). However, the study was compromised.
870.5395	*In vivo* mammalian cytogenetics - erythrocyte micronucleus assay in mice	41710011 (1992) Acceptable/guideline Doses up to 1600 mg/kg	Mice bone marrow - No increase in micronucleated polychromatic erythrocytes occurred with CGA-1 69374 (91.2% a.i).
870.5550	Unscheduled DNA Synthesis in Mammalian Cells in Culture	4210012 (1992) Acceptable/ guideline Doses up to 50 µg/mL	CGA-i69374 tech. (92.2% a.i.) was considered to be negative in the unscheduled DNA synthesis assay in rat primary hepatocytes as measured by an autoradiographic method at concentrations up to 50.0 µg/mL.
870.5550	Unscheduled DNA Synthesis in Mammalian Cells in Culture	42090027 (1985) Unacceptable/ guideline 0.25-31.25 µg/mL	No conclusion can be reached from the unscheduled DNA synthesis (UDS) primary rat hepatocyte assay conducted with difenoconazole technical at concentrations ranging from 0.25 to 31.25 µg /mL. The sensitivity of the study was severely compromised.
870.5550	Unscheduled DNA Synthesis in Mammalian Cells in Culture	42090026 (1985) Unacceptable/ guideline 0.08-10 µg/mL	No conclusion can be reached from the unscheduled DNA synthesis (UDS) human fibroblast assay conducted with difenoconazole tech. at conc. ranging from 0.08 to 10 µg /mL.
870.6200a	Acute neurotoxicity screening battery	46950327 (2006) Acceptable/ guideline 0, 25, 200 or 2000 mg/kg/d	NOAEL (M) = 25 mg/kg/day LOAEL (M) = 200 mg/kg/day based on reduced fore-limb grip strength in males on day 1 and increased motor activity on Day 1. NOAEL (F) = 200 mg/kg/day LOAEL (F) = 2000 mg/kg/day based on decreased body weight, the following clinical signs: upward curvature of the spine, tip-toe gait, decreased activity, piloerection and sides pinched in and decreased motor activity.

Table A.1.2 Subchronic, Chronic and Other Toxicity Profile of Difenoconazole

Guideline No.	Study Type	MRID No. (year)/ Classification /Doses	Results
870.6200b	Subchronic neurotoxicity screening battery	46950329 (2006) Acceptable/ guideline 0, 40, 250, or 1500 ppm M; 0, 2.8, 17.3 or 107.0 mg/kg/d F: 0, 3.2, 19.5, or 120.2 mg/kg/d	NOAEL (M) = 40 ppm (2.8 mg/kg/day) LOAEL (M) = 250 ppm (17.3 mg/kg/day) based on decreased hind limb strength. NOAEL (F) = 250 ppm (19.5 mg/kg/day) LOAEL (F) = 1500 (120.2 mg/kg/day) based on decreased body weight, body weight gain and food efficiency.
870.7485	Metabolism and pharmacokinetics (rat)	42090023 (1990) Acceptable/ guideline 14 daily doses of 0.5 or 300 mg/kg	The absorption, distribution, metabolism, and excretion of CGA 169374 were studied in groups of male and female Sprague-Dawley rats. Animals were administered a single oral gavage dose of 0.5 or 300 mg/kg [^{14}C]CGA- 169374, or 0.5 mg/kg unlabeled GGA-169374 by gavage for 14 days followed by a single gavage dose of 0.5 mg/kg [^{14}C)CGA-169374 on day 15. The test compound was labeled with C^{14} at either the phenyl or triazole ring.
870.7485	Metabolism and pharmacokinetics (rat)	42090031 (1988) Acceptable/ guideline 0.5 or 300 mg/kg	These studies indicate that distribution, metabolism, and elimination of CGA-169374 were not sex related. There was a slight dose difference in the metabolism and elimination of CGA-169374. In phenyl and triazole labeling studies, fecal excretion of radioactivity was higher in the high dose animals compared to the low dose animals, and an additional metabolite was found in the feces of the high dose animals compared to the low dose animals. There was no major difference in the distribution and excretion of radioactivity with labeling at the phenyl and triazole ring positions, however, there were some different metabolites identified. The studies also showed that administration of 0.5 and 300 mg/kg CGA- 169314 did not induce any treatment related clinical effects.
870.7485	Metabolism and pharmacokinetics (rat)	420710013, 42710014 (1990) Acceptable/ guideline 0.5 or 300 mg/kg	These two studies described the absorption, distribution, and excretion as the pharmacokinetics and isolated and identified urinary metabolites. Issues raised in the previous supplementary studies were answered. In conjunction with these studies, the previous studies are upgraded.

Table A.1.2 Subchronic, Chronic and Other Toxicity Profile of Difenoconazole			
Guideline No.	**Study Type**	**MRID No. (year)/ Classification /Doses**	**Results**
870.7485	Metabolism and pharmacokinetics (rat)	42090029 (1987) Acceptable/ guideline	[14C]CGA-169374 was rapidly and extensively distributed. metabolized, and excreted in rats for all dosing regimens. The extent of absorption is undetermined pending determination of the extent of biliary excretion. The 4-day recoveries were 97.4-107.75% of the administered dose for all dosing groups. The elimination of radioactivity in the feces (78.06- 94.61% of administered dose) and urine (8.48-21.86%) were almost comparable for all oral dose groups, with slightly higher radioactivity found in the feces of the high dose group than the low dose groups. This was probably due to biliary excretion, poor absorption or saturation of the metabolic pathway. The radioactivity In the blood peaked at about 24-48 hours for all dosing groups. Half-lives of elimination appear to be approximately 20 hours for the low dose groups and 33 - 48 hours for the high dose group. The study results also indicate that CGA-1 69374 and/or its metabolites do not bioaccumulate to an appreciable extent following oral exposure since all the tissues contained negligible levels (<1%) of radioactivity 7 days postexposure.
870.7485	Metabolism and pharmacokinetics (rat)	42090030 (1987) Acceptable/ guideline	The metabolism of CGA-169374 appears to be extensive because the metabolites accounted for most of the recovered radioactivity in the excreta. Three major metabolites were identified in the feces (i.e., A, B, and C). Two of the metabolites were separated into isomers (i.e., A1, A2, B1, and B2). Metabolite C was detected only In the high dose groups, indicating that metabolism of CGA-169374 is dose related and involves saturation of the metabolic pathway. Free triazole metabolite was dete6ted in the urine of triazole labeled groups and its byproduct was detected In the liver of phenyl labeled groups only. Other urinary metabolites were not characterized.

Table A.1.3. Subchronic, Chronic and Other Toxicity Profile of Difenoconazole Metabolites			
Guideline No.	**Study Type**	**MRID No. (year)/ Classification /Doses**	**Results**
870.5100	*In vitro* Bacterial Gene Mutation *(Salmonella typhimurium/ E. coli)/* mammalian activation gene mutation assay	46950314 (1991) *Unacceptable/ guideline* 0, 31.3, 62.5, 125, 250, 500 or 1000 µg/plate in strains TA100 and TA1537 (-S9) 0, 31.3, 62.5, 125, 250, 500 or 1000 µg/plate in all strains (+S9) 0, 62.5, 125, 250, 500, 1000 or 2000 µg/plate in strains TA1535, WP2 *uvrA* and TA98 (-S9) 0, 62.5, 125, 250, 500, 1000 or 2000 µg/plate in strains WP2 *uvrA* (+S9)	The number of revertants per plate was not increased over the concurrent solvent control value at any test *material concentration, with or without* S9-mix, *in* any tester strain. The solvent and positive controls induced the appropriate responses in the corresponding strains. There was no evidence of induced mutant colonies over background. Tested: CGA-189138 (metabolite of difenoconazole)
870.5100	*In vitro* Bacterial Gene Mutation *(Salmonella typhimurium/ E. coli)/* mammalian activation gene mutation assay	*46950315 (1991)* Unacceptable/ guideline 0, 156, 313, 625, 1250, 2500 or 5000 µg/plate (±S9)	The number of revertants per plate was not increased over the concurrent solvent control value at any test material concentration, with or without S9-mix, in any tester strain. The solvent and positive controls induced the appropriate responses in the corresponding strains. There was no evidence of induced mutant colonies over background. Tested: CGA205374 (metabolite of difenoconazole)
870.5100	*In vitro* Bacterial Gene Mutation *(Salmonella typhimurium/ E. coli)/* mammalian activation gene mutation assay	46950317 (1991) Unacceptable/ guideline 0, 2.50, 5.00, 10.0, 20.0, 40.0 or 80.0 µg/plate *in* all strains (-S9) 0, 5.00, 10.0, 20.0, 40.0, 80.0 or 160 µg/plate in strains TA100 and TA1535 (+S9) 0, 10.0, 20.0, 40.0, 80.0, 160 or 320 µg/plate in strains WP2 *uvrA* and TA1537 (-S9) 0, 2.50, 5.00, 10.0, 20.0, 40.0, or 80.0 µg/plate in strain TA98 (+S9)	The number of revertants per plate was not increased over the concurrent solvent control value at any test material concentration, with or without S9-mix, in any tester strain. The solvent and positive controls induced the appropriate responses in the corresponding strains. **There was no evidence of induced mutant colonies over background.** Tested: CGA205375 (metabolite of difenoconazole)

EXECUTIVE SUMMARIES OF NEW STUDIES:

STUDY TYPE: 28-Day Dermal Toxicity – Rat
(OPPTS 870.3200 [§82-2] (rodent); OECD 410).

CITATION: Gerspach, R. Difenoconazole: 28-Day Repeated Dose Dermal Toxicity Study in Rats. Novartis Crop protection AG Toxicology (Switzerland). Novartis Report Number: 993072; Syngenta Report Number: T002728-06. July 11, 2000, MRID 46950310 and MRID 46950311. Unpublished.

EXECUTIVE SUMMARY: In a 28-day dermal toxicity study (MRID 46950310) CGA 169374 Technical (91.8% a.i., Batch No. P807002) was applied to the shaved skin of ten male and ten female rats at dose levels of 0, 10, 100 and 1000 mg/kg bw/day. There were no treatment-related effects on body weight or food consumption. Non clinical signs of toxicity were noted including specific indicators of neurotoxicity. The dose level of 1000 mg/kg bw/day caused hyperkeratosis at the skin application site. A high incidence of follicular cell hypertrophy of the thyroid was observed in males and females of control and all treatment groups and variations with dose are not considered treatment-related. Minimal inconsequential changes were noted on clinical chemistry parameters in high dose males that were not relevant toxicologically. The incidence and severity was increased in animals in the highest dose group. There was an increase in the absolute (12%) and relative (16%) weight of the liver in males in the high dose group accompanied by an increased incidence of slight hepatocellular hypertrophy (7/10) compared to controls (2/10). Females in the high dose group also had an increase in the relative weight of the liver (10%) with an increased incidence of slight hepatocellular hypertrophy (7/10) compared to controls (1/10). These effects are consistent with adaptive responses of the liver.

A systemic LOAEL for male and female rats was not established. The NOAEL for male and female rats is 1000 mg/kg bw/day.

A dermal irritation LOAEL for male and female rats is 1000 mg/kg bw/day based on hyperkeratosis at the skin application site. The dermal NOAEL for male and female rats is 100 mg/kg bw/day.

This 28-day dermal toxicity study in the Fischer 344 rat is **Acceptable/Guideline** and satisfies the guideline requirement for a 28-day dermal toxicity study (OPPTS 870.3200; OECD 410) in the rat.

STUDY TYPE: Acute Neurotoxicity - Rats OPPTS 870.6200a [§81-8]; OECD 424.

CITATION: Pinto, P.J. (2006) Difenoconazole Technical (CGA169374): Acute Neurotoxicity Study in Rats. Central Toxicology Laboratory (Cheshire, UK). Laboratory report number AR7517-REG-R1, July 28, 2006. MRID 46950327. Unpublished.

Pinto, P.J. (2006) Difenoconazole Technical (CGA169374): Preliminary Acute

Neurotoxicity Study in Rats. Central Toxicology Laboratory (Cheshire, UK). Laboratory report number AR7518-REG, June 16, 2006. MRID 46950325. Unpublished.

EXECUTIVE SUMMARY: In an acute neurotoxicity study (MRID 46950327), groups of fasted Alpk:AP$_f$SD Wistar-derived rats (10/sex/dose), at least 42 days old, were given a single oral dose of difenoconazole technical (CGA169374) (94.3% w/w, batch/lot # WM806228) in 1% w/v aqueous carboxymethylcellulose (CMC) at doses of 0, 25, 200, or 2000 mg/kg bw and observed for 14 days. Dose levels selected for this study were based on the results of a preliminary acute neurotoxicity study (MRID 46950325). Neurobehavioral assessment (functional observational battery and motor activity testing) was performed on 10 animals/sex/group on days -7, 1, 8, and 15. Body weight and food consumption were measured weekly throughout the study. At study termination, 5 animals/sex/group were euthanized and perfused *in situ* for neuropathological examination; brain weight was recorded from these animals. Of the perfused animals, 5 animals/sex from the control and high dose groups were subjected to histopathological evaluation of brain and peripheral nervous system tissues.

There were no unscheduled deaths at any dose level. Weight change on the day of dosing by the control, low-, mid-, and high-dose groups was -2.1, -1.0, -7.8, and -18.3 g, respectively, for males and 0.0, 2.1, -3.8, and -13.0 g, respectively, for females. Body weight for females had recovered to control levels by day 8. Food consumption for males given 2000 mg/kg was approximately 20% less than control during week 1 only ($p<0.01$). Food consumption for these animals recovered to control levels during week 2. There were no differences from control for females at any dose level or for males at the lower dose levels. These effects on body weight and food consumption were not toxicologically significant.

At 2000 mg/kg, a number of adverse clinical signs were observed on day 1 (at the time of peak effect), including: upward curvature of the spine (8 males, 9 females); tip-toe gait (3, 8); decreased activity (6, 7); piloerection (3, 5); sides pinched in (3, 7); and subdued (1, 0). Females were affected more than males. All treatment-related clinical signs observed on day 1 showed complete recovery by day 5 (males) or day 7 (females).

Significant decreases in fore-limb grip strength were seen in mid- (↓23%) and high-dose (↓26%) males on day 1. Females dosed with 2000 mg/kg had lower motor activities on day 1 (37%), at the time of peak effect, and on day 8 (31%). Males dosed with 200 or 2000 mg/kg had higher motor activities than the controls on day 1, 50% and 55%, respectively, at the time of peak effect.

There were no effects on brain weight at any dose level. Neuropathological examination of the central and peripheral nervous system showed no effects of treatment at doses of 2000 mg/kg in both sexes.

The LOAEL for acute neurotoxicity of difenoconazole technical (CGA169374) in male rats is 200 mg/kg bw based on reduced fore-limb grip strength in males on day 1 and increased motor activity on Day 1. The NOAEL is 25 mg/kg bw.

The LOAEL for acute neurotoxicity of difenoconazole technical (CGA169374) in female

rats is 2000 mg/kg bw based on decreased body weight, the following clinical signs: upward curvature of the spine, tip-toe gait, decreased activity, piloerection and sides pinched in and decreased motor activity. The NOAEL is 200 mg/kg bw.

This acute neurotoxicity study is classified as **Acceptable/Guideline** and satisfies the guideline requirement for an acute neurotoxicity study in rats (870.6200; OECD 424). Positive control data have been submitted for review and were considered acceptable.

STUDY TYPE: Subchronic Neurotoxicity, OPPTS 870.6200b [§82-7] feeding - rat; (OECD 424).

CITATION: Pinto, P J. (2006). Difenoconazole technical (CGA 169374) subchronic neurotoxicity study in rats, final report. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK SK 10 4TJ. Report number PR1330-REG-R1. July 28, 2006. MRID 46950329. Unpublished.

Pinto, P.J. (2006). Difenoconazole technical (CGA 169374) 28-day dietary rangefinding study in rats, final report. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK SK 10 4TJ. Report number KR1606-REG. June 13, 2006. MRID 46950326. Unpublished.

Alexander, O. (2006) Difenoconazole technical (CGA 169374) subchronic neurotoxicity study in rats – study profile. Syngenta Crop Protection, Inc., 410 Swing Road, P.O. Box 18300, Greensboro, NC 27419-8300. Report number PR1330-REG-R1. September 19, 2006. MRID 46950330. Unpublished.

EXECUTIVE SUMMARY: In a subchronic neurotoxicity study (MRID 46950329) difenoconazole technical (94.5% w/w, batch no. WM806228) was administered to groups of 12 male and 12 female Alpk:AP$_f$SD (Wistar-derived) rats at concentrations of 0, 40, 250, or 1500 ppm in the diet for 90 days. Respective dose levels corresponded to 0, 2.8, 17.3 or 107.0 mg/kg bw/day for males and 0, 3.2, 19.5, or 120.2 mg/kg bw/day for females. Neurobehavioral assessment (functional observational battery and motor activity testing) was performed in 12 animals/sex/group pretest and during weeks 2, 5, 9, and 14. Cholinesterase activity was not determined. At study termination, 5 animals/sex/group were euthanized and perfused *in situ* for neuropathological examination. Of the perfused animals, 5/sex from the control group and 5/sex from the 1500 ppm group were subjected to histopathological evaluation of brain and peripheral nervous system tissues.

Treatment with difenoconazole at concentrations up to 1500 ppm in the diet had no effect on mortality or clinical signs. Relative to respective control weight, final body weight of males and females in the 1500 ppm group was reduced by 9% and 7%. Body weight gain was reduced by 22% in males and 23% in females. Food consumption was reduced in this group (statistically significant only in females [7%]), and food efficiency was significantly reduced in males by 21% ($p \leq 0.05$) and in females by 21% (ns). Lower dose groups were unaffected. Absolute liver weight in males and females in the 1500 ppm group was increased over respective control weight by 38% and 45%. Liver was not weighed in lower dose groups. The increase in liver weight was

considered a normal response to chemical treatment.

During weeks 2, 9 and 14, hind-limb grip strength in males in the 1500 ppm group was reduced by 18 to 27% relative to the control values. At week 14, hind-limb grip strength in males in the 250 ppm group was significantly ($p \leq 0.05$) reduced by 20% relative to the control values. FOB observations in females were unaffected by treatment. Motor activity was unaffected in both sexes at all observation times. Brain weight was unaffected by treatment and there were no treatment-related neuropathological lesions.

The LOAEL in male rats is 250 ppm in the diet (17.3 mg/kg bw/day), based on decreased hind limb strength. The NOAEL is 40 ppm (2.8 mg/kg bw/day).

The LOAEL in female rats is 1500 ppm in the diet (120.2 mg/kg bw/day), based on decreased body weight, body weight gain and food efficiency. The NOAEL is 250 ppm (19.5 mg/kg bw/day).

The study is classified as **Acceptable/Guideline** and does satisfies the guideline requirement for a subchronic neurotoxicity study in rats (870.6200b). Positive control data have been submitted for review and were considered acceptable.

STUDY TYPE: Rodent *In Vivo* Dermal Penetration Study – Rat
OPPTS 870.7600 [§85-2]; OECD none.

CITATION: Hassler, S. (2003) Difenoconazole 250 EC (A7402G): Dermal absorption of [Triazole-U-^{14}C] CGA 169374 formulated as Score® 250 EC (A-7402G) in the rat (*in vivo*). Syngenta Crop Protection AG, Health Assessment/Animal Metabolism CH-4002 Basel, Switzerland. Syngenta Number T002729-06, May 6, 2003. MRID 46950333. Unpublished.

EXECUTIVE SUMMARY: In a dermal penetration study (MRID 46950333), [Triazole–U-^{14}C] CGA 169374 (radiolabled: batch # 50.2-1 and 50.2-2 contained 98 and 99.3% a.i., respectively; nonradiolabeled: batch # AMS 255/3 contained 99.3% a.i.) formulated as Score® 250 EC (A-7402G) was administered to 16 male HanBrl: WIST (SPF) rats/dose to a skin area of 10 cm^2 at nominal dose levels of 0, 0.005, 0.0125, and 2.5 mg/cm^2 skin. The 2.5 mg/cm^2 dose was repeated because of a high variability in the results of the washing procedure. Measured dose levels were 0.005, 0.0130, 2.4, and 2.6 mg/cm^2 for the low, mid, and high dose and high dose repeat groups, respectively. Exposure duration was 6 hours and animals were monitored for 6, 24, 48, or 72 hours. The remaining discussion of dermal penetration at the high dose will include only the "high-dose repeat" data (i.e., the results of the first high-dose exposure will not be discussed). Recovery of the applied dose was acceptable with group means ranging from 95.44 to 103.67%. Results were not adjusted for incomplete recovery of the applied dose. The majority of the applied dose was recovered in the skin wash, accounting for 49-69%, 73-78%, and 76-86% of the low, mid, and high dose, respectively. The amount of the applied dose retained at the application site was 8-12%, 3-5%, and 2-5% of the low, mid, and high dose, respectively. At the low and mid dose, the major part of the radioactivity remaining in the skin was associated with the *stratum corneum* (7-11% and 2-5%, respectively), while only 1-2% of

the high dose was recovered in the upper skin layer. Dermal absorption (sum of blood, carcass, urine, feces, skin test site, gastrointestinal tract, untreated skin, and cagewash) accounted for 15-38%, 7-15%, and 3-11% of the low, mid, and high doses, respectively. Of the test substance systemically absorbed, excretion into the feces was generally the primary route of elimination, accounting for up to 18%, 8%, and 2% of the low, mid, and high doses, respectively. Of the radioactivity remaining in the animal 72 hours after application, the gastrointestinal tract contained 3.0%, 1.4%, and 0.3% of the low, mid, and high doses, respectively, and the carcass contained 1.5%, 0.7%, and 1.1%, respectively. Blood concentrations during and after the exposure period were at or below the limits of determination. Based on the limited blood concentration data available, maximum blood concentrations were measured between 6-8 hours after dose application.

Based on the amount of radioactivity entering the systemic circulation within 6 hours of exposure, the calculated penetration rates at the low, mid, and high doses were 0.013, 0.162, and 30.4 $\mu g\ cm^{-2}\ h^{-1}$, respectively. The penetration rates increased somewhat proportionally with the increase of the test substance concentration at the three dose levels (1:26:5100 for the concentration ratio of the dose levels versus 1:12:2300 for the ratio of the penetration values).

This study in the rat is **unacceptable/guideline** and does not satisfy the guideline requirement for a dermal penetration study (870.7600) in rats. Major deficiencies include uncertainty in the ability of the laboratory to perform the experiment, and only one exposure duration was tested (6 hours), despite minimum Guideline recommendations for durations of 1, 10, and 24 hours. See "Study Deficiencies" for listing of numerous minor deficiencies.

STUDY TYPE: ***In Vitro* Dermal Penetration Study – Rat and Human**
OPPTS 870.7600 [§85-2]; OECD none.

CITATION: Hassler, S. (2003) Difenoconazole 250 EC (A7402G): The percutaneous penetration of [Triazole-U-^{14}C] CGA 169374 formulated as Score® 250 EC (A-7402G) through rat and human split-thickness skin membranes (*in vitro*). Syngenta Crop Protection AG, Health Assessment/Animal Metabolism CH-402 Basel, Switzerland. Syngenta Number T002730-06, April 9, 2003. MRID 46950332. Unpublished.

EXECUTIVE SUMMARY: In an *in vitro* percutaneous penetration study (MRID 46950332), [Triazole–U-^{14}C] CGA 169374 (98% a.i., batch number 50.2-1) mixed with nonradiolabeled CGA 169374 (batch number AMS 255/3 containing 99.3% a.i.) formulated as SCORE 250® (A-7402) was applied to skin membranes prepared from rat [male HanBrl: WIST (SPF)] and human (cadaver) abdominal skin. Percutaneous absorption at low, mid, and high doses of 0.5, 12.5, or 2500 $\mu g/cm^2$ (actual applied doses of 0.5, 12, or 2345 $\mu g/cm^2$) was assessed over 24 hours.

Results clearly indicate that transfer of [Triazole–U-^{14}C] CGA 169374 across skin membrane was notably greater for the rat skin membrane than for human skin membrane as shown by flux values that were 10-, 12-, and 32-fold greater for the low, mid, and high concentrations, respectively. A concentration-dependent absorption was also indicated by greater flux values with increasing concentration: flux values at the low, mid, and high doses for the rat skin

membranes were 0.020, 0.455, and 26.2 ug/cm^2, respectively, and for human skin membranes were 0.002, 0.037, and 0.822 ug/cm^2, respectively. The increasing flux values resulted in greater absolute amounts of test article being transferred across the skin membranes with increasing concentration: values at the low, mid, and high doses for the rat skin membranes were 0.35, 7.7, and 539.2 ug/cm^2, respectively, and for human skin membranes were 0.04, 0.84, and 15.6 ug/cm^2, respectively. However, the percutaneous absorption was decreased, indicating saturated kinetics (absorption values at the low, mid, and high doses expressed as percent of applied dose for the rat skin membranes were 71%, 64%, and 23%, respectively, and for human skin membranes were 8%, 7%, and 0.7%, respectively).

This *in vitro* percutaneous absorption study in the rat is **acceptable/nonguideline**, but does not satisfy the guideline requirement for a dermal penetration study (870.7600) in rats. The study is a specialty study and was designed to provide only supplemental information to the OPPTS 870.7600 requirement. Results of this study provide information on the differences in dermal absorption between rat and human skin membranes.

STUDY TYPE: *In vitro* Mammalian Cytogenetics (chromosomal aberrations) assay in Chinese hamster CHO cells; OPPTS 870.5375 [§84-2]; OECD 473

CITATION: Lloyd, M. (2001) Difenoconazole Technical: Induction of chromosome aberrations in cultured Chinese hamster ovary (CHO)cells. Covance Laboratories Ltd., Otley Road, Harrogate, North Yorkshire HG3 1PY, England. Laboratory Project ID: Covance Number 252/293, Syngenta Number T002874-06, December 11, 2001. MRID 46950319. Unpublished

EXECUTIVE SUMMARY: In a mammalian cell cytogenetics assay (Chromosomal aberrations) (MRID 46950319), Chinese hamster CHO cells in culture were exposed to CGA 169374 Technical (94.3% w/w, Lot No. WM806228) in DMSO for three hours at concentrations of 0, 21.99, 27.49, or 34.36 µg/mL without metabolic activation (S9-mix) and at concentrations of 0, 34.36, 53.69 or 67.11 µg/mL with S9-mix. Cells were harvested 17 hours following the end of exposure. Cells were exposed in a second confirmatory study for three hours at concentrations of 0, 21.99, 27.49 or 34.36 µg/mL without S9-mix and for three hours at concentrations of 0, 34.36, 53.69, 67.11 or 83.89 µg/mL with S9-mix. Cells were harvested 17 hours following exposure. Cells were evaluated for the presence of structural chromosomal aberrations and for numerical aberrations (polyploidy, endoreduplication and hyperploidy). The S9-fraction was obtained from Aroclor 1254 induced male Sprague-Dawley rat liver.

CGA 169374 Technical was tested up to cytotoxic concentrations as evidenced by a dose-related reduction in mitotic activity seen with and without S9-mix. There was a statistically significant increase in the percentage of cells with structural chromosomal aberrations at a CGA 169374 Technical concentration of 34.36 µg/mL without S9-mix in the first study. The slides were rescored to determine if aberrations at the fragile X site were present. Aberrations at the fragile X site are not likely relevant to clastogenicity. Aberrations at the fragile X site were not found but the values obtained on rescoring were within the historical solvent control range. There was no clear reason given why the percent of aberrant cells was lower when the slides were rescored.

Possibly the distribution of cells on the slides was uneven. The increase at this dose without S9-mix was not seen in the confirmatory assay and thus the increase was not considered biologically significant. A statistically significant increase in the percent of aberrant cells was seen in the first study at 67.11 µg/mL with S9-mix. The statistical significance remained upon rescoring and all values exceeded the historical solvent control range. No statistically significant increase was seen at this or a higher concentration in the confirmatory study with S9-mix. The failure to see a significant increase in the percent of aberrant cells in the confirmatory study makes the results equivocal. The solvent and positive controls (4-Nitroquinoline 1-oxide without S9-mix and cyclophosphamide with S9-mix) induced the appropriate responses. **There was evidence of a weak induction of structural chromosomal aberrations over background in the presence of S9-mix.**

This study is classified as **Acceptable/Guideline** and satisfies the guideline requirement for *OPPTS 870.5375; OECD 473* for *in vitro* cytogenetic mutagenicity data.

STUDY TYPE: ***In vitro*** **Mammalian Cytogenetics (chromosomal aberrations) assay in Chinese hamster CHO cells; OPPTS 870.5375 [§84-2]; OECD 473**

CITATION: Ogorek, B. (2001) Difenoconazole Technical: Cytogenetic test on Chinese hamster cells *in vitro*. Syngenta Crop Protection AG, Health Assessment 2 Stein/Genetic Toxicology, CH-4332 Stein, Switzerland. Laboratory Project ID: Syngenta AG Test Number 20013013, Syngenta Number T002875-06, December 3, 2001. MRID 46950321. Unpublished

EXECUTIVE SUMMARY: In a mammalian cell cytogenetics assay (Chromosomal aberrations) (MRID 46950321), Chinese hamster CHO cells in culture were exposed to CGA 169374 Technical (94.3% w/w, Lot No. WM806228) in DMSO for three hours at concentrations of 0, 26.3, 39.5 or 59.3 µg/mL without metabolic activation (S9-mix) and at concentrations of 0, 11.7 or 17.6 µg/mL with S9-mix. Cells were harvested 18 hours following the end of exposure. Cells were exposed in a second confirmatory study for 21 hours at concentrations of 0, 2.3, 5.2 or 11.7 µg/mL without S9-mix and for three hours at concentrations of 0, 7.8, 11.7 or 17.6 µg/mL with S9-mix. Cells were harvested immediately following the 21-hour exposure and 18 hours after the three-hour exposure. Cells were evaluated for the presence of structural chromosomal aberrations and for polyploidy. The S9-fraction was obtained from Aroclor 1254 induced male HanIbm:WIST(SPF) rat liver.

CGA 169374 Technical was tested up to cytotoxic concentrations as evidenced by a dose-related reduction in mitotic activity seen with and without S9-mix. There was a statistically significant increase in the percentage of CHO cells with structural chromosomal aberrations at a CGA 169374 Technical concentration of 59.3 µg/mL without S9-mix in the original study when aberrations at the fragile X site were included but not when they were excluded. Aberrations at the fragile X site are not likely relevant to clastogenicity. No statistically significant increase in the percent of aberrant cells was seen in the original study with S9-mix. An increase in the percent of aberrant cells was seen in the confirmatory study at 17.6 µg/mL with S9-mix and the increase was statistically significant ($p \leq 0.001$) when aberrations at the fragile X site were excluded. The value of 6.5% aberrant cells exceeded the value of >6% set as a criterion for a

positive effect in the testing laboratory. The failure to see a significant increase in the percent of aberrant cells in the original study makes the results equivocal. No statistically significant increase in the percentage of aberrant cells was seen at any of the three test material concentrations without S9-mix in the confirmatory study. The solvent and positive controls (Mitomycin C without S9-mix and Cyclophosphamide with S9-mix) induced the appropriate responses. **There was evidence of a weak induction of structural chromosomal aberrations over background.**

This study is classified as **Acceptable/Guideline** and satisfies the guideline requirement for *OPPTS 870.5375; OECD 473* for *in vitro* cytogenetic mutagenicity data.

STUDY TYPE: ***In vitro*** **Mammalian Cytogenetics (chromosomal aberrations) assay in human lymphocytes; OPPTS 870.5375 [§84-2]; OECD 473**

CITATION: Fox, V. (2001) Difenoconazole Technical: *In* vitro cytogenetic assay in human lymphocytes. Central Toxicology Laboratory, Alderley Park/Macclesfield, Cheshire, UK SK10 4TJ. Laboratory Project ID: CTL Number SV1090, Syngenta Number T002876-06, August 29, 2001. MRID 46950323. Unpublished

EXECUTIVE SUMMARY: In a mammalian cell cytogenetics assay (Chromosomal aberrations) (MRID 46950323), human lymphocytes in culture were exposed to CGA 169374 Technical (94.3% w/w, Lot No. WM806228) in DMSO for three hours at concentrations of 0, 5, 30 or 75 µg/mL without metabolic activation (S9-mix) and at concentrations of 0, 5, 30 or 62 µg/mL with S9-mix. Cells were harvested 17 hours following the end of exposure. Cells were exposed in a second experiment for 20 hours at concentrations of 0, 1, 5 or 10 µg/mL without S9-mix and for three hours at concentrations of 0, 5, 30 or 50 µg/mL with S9-mix. Cells were harvested immediately following the 20-hour exposure and 17 hours after the three-hour exposure. Cells were evaluated for the presence of structural chromosomal aberrations. The S9-fraction was obtained from Phenobarbital + β-naphthoflavone induced male Sprague-Dawley rat liver.

CGA 169374 Technical was tested up to cytotoxic concentrations as evidenced by a dose-related reduction in mitotic activity seen with and without S9-mix. No statistically significant increases in the percentage of cells with structural aberrations, excluding gaps, over the solvent control values were seen at any test material concentration with or without S9-mix in the first experiment or without S9-mix in the second experiment. A statistically significant increase over the solvent control value was seen at 5µg/mL with S9-mix in the second experiment; however, the increase was not considered biologically significant because the value (4.00%) was within the historical solvent control range, the values at the two higher concentrations were not significantly increased and no increase was seen in the first experiment. The solvent and positive controls (Mitomycin C without S9-mix and Cyclophosphamide with S9-mix) induced the appropriate responses. **There was no evidence of structural chromosomal aberrations induced over background.**

This study is classified as **Acceptable/Guideline** and satisfies the guideline requirement for *OPPTS 870.5375; OECD 473* for *in vitro* cytogenetic mutagenicity data.

DIFENOCONAZOLE METABOLITES:

STUDY TYPE: *In vitro* Bacterial Gene Mutation (Bacterial system, *Salmonella typhimurium* and *Escherichia coli*)/ mammalian activation gene mutation assay; OPPTS 870.5100 [§84-2]; OECD 471 (formerly OECD 471 & 472).

CITATION: Nakajima, M. (1991) CGA189138 (metabolite of difenoconazole): reverse mutation assay of CGA189138. Biosafety Research Center; Foods, Drugs and Pesticides (An-Pyo Center); 582-2, Arahama Shioshinden; Fukude-Cho Iwata-Gun.; Shizuoka 437-12; Japan. Laboratory Project ID: BRC Number 1809, October 21, 1991. MRID 46950314. Unpublished.

EXECUTIVE SUMMARY: In a reverse gene mutation assay in bacteria (MRID 46950314), strains TA98, TA100, TA1535 and TA1537 of *S. typhimurium* and strain WP2 *uvrA of E. coli* were exposed to CGA-189138, a metabolite of difenoconazole, (97.8% a.i., lot number 910806) dissolved in DMSO in two independent assays using a 20-minute preincubation procedure and duplicate plating. In the first mutagenicity assay, which was alternatively called the pilot assay and the dose-finding assay, concentrations of 0, 51.2, 128, 320, 800, 2000 or 5000 µg/plate were tested with and without S9-mix. In the second assay, which was called the main assay, concentrations of 0, 31.3, 62.5, 125, 250, 500 or 1000 µg/plate were tested in the absence of S9-mix in strains TA100 and TA1537 and in the presence of S9-mix in all *Salmonella* strains; concentrations of 0, 62.5, 125, 250, 500, 1000 or 2000 µg/plate were tested in the absence of S9-mix in strains TA1535, WP2 *uvrA* and TA98 and in the presence of S9-mix in strain WP2 *uvrA*. The S9 fraction was obtained from phenobarbital and 5,6-benzoflavone-induced male Sprague-Dawley rat liver.

CGA-189138 was tested at concentrations up to the limit concentration for the assay in the pilot assay, and many of the higher concentrations tested in both assays showed cytotoxicity and sometimes also insolubility. In the absence of S9-mix in the pilot assay, the test material was cytotoxic, as judged by stereomicroscopic examination of the bacterial lawns, at concentrations of 800 µg/plate and higher in strains TA100 and TA1537 and at concentrations of 2,000 µg/plate and higher in strains TA1535, WP2 *uvrA* and TA98. In the presence of S9-mix in the pilot assay, the test material was cytotoxic at concentrations of 800 µg/plate and higher in all four *Salmonella* strains and at concentrations of 2,000 µg/plate and higher in strain WP2 *uvrA*. In the absence of S9-mix in the main assay, the test material was cytotoxic at concentrations of 500 µg/plate and higher in strain TA100, at concentrations of 1,000 µg/plate and higher in strains TA1535 and TA98, and at the maximum concentrations tested in strains WP2 *uvrA* and TA1537. In the presence of S9-mix in the main assay, the test material was cytotoxic at concentrations of 500 µg/plate and higher in strains TA100, TA1535 and TA1537 and at the maximum concentrations tested in strains WP2 *uvrA* and TA98. At cytotoxic concentrations there was often also a marked decrease in the number of revertant colonies found. In the pilot assay, precipitation of the white powdery test material was observed on the surface of the agar plates at the time of colony counting at concentrations of 5,000 µg/plate in the absence of S9-mix and at concentrations of 2,000 µg/plate and above in the presence of S9-mix. In the main assay, such precipitation was observed only in the presence of S9-mix and at the highest concentration tested in strain WP2 *uvrA*. The number of revertants per plate was not increased over the concurrent solvent control

value at any test material concentration, with or without S9-mix, in any tester strain. The solvent and positive controls induced the appropriate responses in the corresponding strains. **There was no evidence of induced mutant colonies over background.**

This study is classified as **Unacceptable/Guideline** and does not satisfies the requirements for Test Guideline OPPTS 870.5100; OECD 471 for *in vitro* mutagenicity (bacterial reverse gene mutation) data. Five strains of *S. typhimurium* were not used in the assay. The study can not be upgraded.

STUDY TYPE: *In vitro* Bacterial Gene Mutation (Bacterial system, *Salmonella typhimurium* and *Escherichia coli*)/ mammalian activation gene mutation assay; OPPTS 870.5100 [§84-2]; OECD 471 (formerly OECD 471 & 472).

CITATION: Nakajima, M. (1991) CGA205374 (metabolite of difenoconazole): reverse mutation assay of CGA205374. Biosafety Research Center; Foods, Drugs and Pesticides (An-Pyo Center); 582-2, Arahama Shioshinden; Fukude-Cho Iwata-Gun.; Shizuoka 437-12; Japan. Laboratory Project ID: BRC Number 1746, August 14, 1991. MRID 46950315. Unpublished.

EXECUTIVE SUMMARY: In a reverse gene mutation assay in bacteria (MRID 46950315), strains TA98, TA100, TA1535 and TA1537 of *S. typhimurium* and strain WP2 *uvrA of E. coli* were exposed to CGA-205374, a metabolite of difenoconazole, (99.3% a.i., lot number 9106054) dissolved in DMSO in two independent assays using a 20-minute preincubation procedure and duplicate plating. In the first mutagenicity assay, which was alternatively called the pilot assay and the dose-finding assay, concentrations of 0, 51.2, 128, 320, 800, 2000 or 5000 µg/plate were tested with and without S9-mix. In the second assay, which was called the main assay, concentrations of 0, 156, 313, 625, 1250, 2500 or 5000 µg/plate were tested with and without S9-mix. The S9 fraction was obtained from phenobarbital and 5,6-benzoflavone-induced male Sprague-Dawley rat liver.

CGA-205374 was tested at concentrations up to the limit concentration for the assay, but the effective concentrations tested were limited by insolubility. Evidence of cytotoxicity, which was collected by stereomicroscopic examination of the bacterial lawns, was seen only in strain TA1537 at the maximum concentration tested, and then only in the main assay in the presence of S9-mix. The test material was quite insoluble, with cloudiness of the preincubation mixture being observed even at 128 µg/plate. The white powdery precipitate of the test material was observed on the surface of the agar plates at the time of colony counting at concentrations of 320 µg/plate and higher in the pilot assay in the absence of S9-mix and at concentrations of 800 µg/plate and higher in the presence of S9-mix. In the main assay, this precipitate was noted at concentrations of 313 µg/plate and higher both in the presence and absence of S9-mix. This precipitate became heavy enough to make it difficult to observe the bacterial lawn at concentrations of 1250 µg/plate or higher in the absence of S9-mix and at the concentration of 5000 µg/plate in the presence of S9-mix. The number of revertants per plate was not increased over the concurrent solvent control value at any test material concentration, with or without S9-mix, in any tester strain. The solvent and positive controls induced the appropriate responses in the corresponding strains. **There was no evidence of induced mutant colonies over**

background.

This study is classified as **Unacceptable/Guideline** and does not satisfies the requirements for Test Guideline OPPTS 870.5100; OECD 471 for *in vitro* mutagenicity (bacterial reverse gene mutation) data. Five strains of *S. typhimurium* were not used in the assay. The study can not be upgraded.

STUDY TYPE: *In vitro* Bacterial Gene Mutation (Bacterial system, *Salmonella typhimurium* and *Escherichia coli*)/ mammalian activation gene mutation assay; OPPTS 870.5100 [§84-2]; OECD 471 (formerly OECD 471 & 472).

CITATION: Nakajima, M. (1991) CGA205375 (metabolite of difenoconazole): reverse mutation assay of CGA205375. Biosafety Research Center; Foods, Drugs and Pesticides (An-Pyo Center); 582-2, Arahama Shioshinden; Fukude-Cho Iwata-Gun.; Shizuoka 437-12; Japan. Laboratory Project ID: BRC Number 1747, August 14, 1991. MRID 46950317. Unpublished.

EXECUTIVE SUMMARY: In a reverse gene mutation assay in bacteria (MRID 46950317), strains TA98, TA100, TA1535 and TA1537 of *S. typhimurium* and strain WP2 *uvrA of E. coli* were exposed to CGA-205375, a metabolite of difenoconazole, (99.8% a.i., lot number 9106055) dissolved in DMSO in two independent assays using a 20-minute preincubation procedure and duplicate plating. In the first mutagenicity assay, which was alternatively called the pilot assay and the dose-finding assay, concentrations of 0, 51.2, 128, 320, 800, 2000 or 5000 µg/plate were tested with and without S9-mix. In the second assay, which was called the main assay, concentrations of 0, 2.50, 5.00, 10.0, 20.0, 40.0 or 80.0 µg/plate were tested in the absence of S9-mix in all strains; concentrations of 0, 5.00, 10.0, 20.0, 40.0, 80.0 or 160 µg/plate were tested in the presence of S9-mix in strains TA100 and TA1535; concentrations of 0, 10.0, 20.0, 40.0, 80.0, 160 or 320 µg/plate were tested in the presence of S9-mix in strains WP2 *uvrA* and TA1537; and concentrations of 0, 2.50, 5.00, 10.0, 20.0, 40.0, or 80.0 µg/plate were tested in the presence of S9-mix in strain TA98. The S9 fraction was obtained from phenobarbital and 5,6-benzoflavone-induced male Sprague-Dawley rat liver.

CGA-205375 was tested at concentrations up to the limit concentration for the assay in the pilot assay. Most of the concentrations in that assay showed cytotoxicity and some of the higher ones also showed insolubility. Some of the higher concentrations in the main assay, which used much lower concentrations, also showed cytotoxicity. In the absence of S9-mix in the pilot assay, the test material was cytotoxic, as judged by stereomicroscopic examination of the bacterial lawns, at all concentrations in strains TA100 and TA1537 and at concentrations of 128 µg/plate and higher in strains TA1535, WP2 *uvrA* and TA98. In the presence of S9-mix in the pilot assay, the test material was cytotoxic at all tested concentrations in strain TA98, at concentrations of 128 µg/plate and higher in strains TA100 and TA1535, and at concentrations of 320 µg/plate and higher in strains WP2 *uvrA* and TA1537. In the absence of S9-mix in the main assay, the test material was cytotoxic at concentrations of 40.0 µg/plate and higher in strain TA100 and at the highest tested concentration in the other strains. In the presence of S9-mix in the main assay, the test material was cytotoxic at concentrations of 40.0 µg/plate and higher in strain TA98, at concentrations of 80.0 µg/plate and higher in strain TA100, at concentrations of 160 µg/plate and

higher in strain TA1537 and at the maximum concentrations tested in strains TA1535 and WP2 *uvrA*. At cytotoxic concentrations there was often also a marked decrease in the number of revertant colonies found. Because cytotoxicity was excessive at most concentrations, the pilot assay provided only slight useful information on mutagenesis in most strains. In the pilot assay, the needle crystalline precipitate of the test material was observed on the surface of the agar plates at the time of colony counting at concentrations of 800 µg/plate and above in the absence of S9-mix and at concentrations of 2,000 µg/plate and above in the presence of S9-mix. No precipitation was observed at any of the much lower concentrations tested in the main assay. The number of revertants per plate was not increased over the concurrent solvent control value at any test material concentration, with or without S9-mix, in any tester strain. The solvent and positive controls induced the appropriate responses in the corresponding strains. **There was no evidence of induced mutant colonies over background.**

This study is classified as **Unacceptable/Guideline** and does not satisfies the requirements for Test Guideline OPPTS 870.5100; OECD 471 for *in vitro* mutagenicity (bacterial reverse gene mutation) data. Five strains of *S. typhimurium* were not used in the assay. The study can not be upgraded.

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	DPD333320
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